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ENVIRONMENTAL RESEARCH

TECHNOLOGY TRANSFER CONFERENCE • 1988

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PROCEEDINGS

SESSION B
WATER QUALITY RESEARCH

November 28 & 29
Royal York Hotel
Toronto, Ontario
Canada

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SESSION B
WATER QUALITY RESEARCH

Sponsored by
Research and Technology Branch
Environment Ontario
Ontario, Canada

Introduction

Environment Ontario holds its annual Technology Transfer Conference to report and publicize the progress made on Ministry-funded projects. These studies are carried out in Ontario Universities and by private research organizations and companies.

The papers presented at Technology Transfer Conference 1988 are published in five volumes of conference Proceedings corresponding to the following sessions:

SESSION A:	AIR QUALITY RESEARCH
SESSION B:	WATER QUALITY RESEARCH
SESSION C:	LIQUID AND SOLID WASTE RESEARCH
SESSION D:	ANALYTICAL METHODS
SESSION E:	ENVIRONMENTAL ECONOMICS

This volume is comprised of presentations given during Session B of the conference.

For reference purposes, indices for sessions A,C,D and E may be found at the back of this volume, listed in alphanumeric order.

For further information on any of the papers, please contact either the authors or the Research and Technology Branch at (416) 323-4574 or 323-4573.

Acknowledgements

The Conference Committee would like to thank the authors for their valuable contributions to environmental research in Ontario.

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KEYNOTE PAPER I

Science-Based Innovation

Science-based innovation is critical in today's global economy to sustain and enhance a nation's prosperity. In seeking to sustain and enhance its prosperity by participating in a growing volume of world trade, large and small economies, face critical problems of adapting their institutions, policies and practices to a radically new environment. Key elements of this environment are that world trade now occurs in a global economy in which the interweaving of science, engineering and technology has acquired the power to transform the comparative advantage and prosperity of nations. With the scale, scope and openness of the international enterprise of science, the transferability of technologies and the mobility of capital, science-based innovation has become a driving force for the technological and corporate change that creates new tradeable goods and services. These conditions are radically different from those of the Industrial Revolution.

In a modern economy the sector which produces tradeable goods and services supported by the first service sector of financial, legal, energy, transportation, communication systems, etc., generates the income that enables a country to invest in the second service sector of education, health care and other personal and social benefits. (Figure 1). In some countries financial institutions have been operating in a manner that hampers the developments in the tradeable goods and services sector.

To participate in the global economy driven by science-based innovation it is essential that, on a national or regional basis, the pyramid of research capacity (in terms of knowledge flow) that leads to tradeable products and services has integrity, that is, that there be a reasonable balance of capacity throughout the pyramid. (Figure 2).

Increasingly science-based innovation requires a strong long-term applied research capacity, particularly in relation to emerging generic technologies, that is industry-based and controlled. This capacity has to be linked to a high quality fundamental research base and a strong market focused development capability.

Large and small countries in different stages of development faces problems in;

i) achieving structural integrity necessary for science-based innovation suitable for their limited resources of people and money, and

ii) using their limited resources effectively.

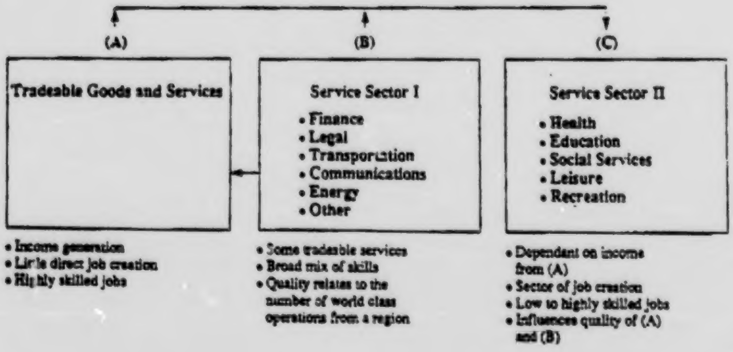

(A)
(B)
(C)
Tradeable Goods and Services
Service Sector I
• Finance
• Legal
• Transportation
• Communications
• Energy
• Other
Service Sector II
• Health
• Education
• Social Services
• Leisure
• Recreation
• Income generation
• Little direct job creation
• Highly skilled jobs
• Some tradeable services
• Broad mix of skills
• Quality relates to the number of world class operations from a region
• Dependant on income from (A)
• Sector of job creation
• Low to highly skilled jobs
• Influences quality of (A) and (B)

Figure 1: A Simple Model of the Economy

In today's global economy it is important to understand the relationship between innovation in the production of tradeable goods and services and the generation of income. A simple model in terms of stating the key issues is given in Figure 1. This model segments the economy into three blocks labeled (A) Tradeable Goods and Services, (B) Service Sector I and (C) Service Sector II. The major source of income which sustains our standard of living comes from sector (A) Tradeable Goods and Services. Canada's current standard of living requires very substantial volumes of trade into world markets. In the globally competitive market of today a nation must be concerned with maintaining and enhancing the environment it creates for business and industry that can innovate in the production of tradeable goods and services. To function effectively, such enterprises require a high quality service sector, namely (B) Service Sector I, comprising such services as finance, legal, energy, construction, communications, transportation, distribution. It is the combination of this business service sector (which produces some tradeable services) and the sector directly producing tradeable goods and services which generate the primary income of a region.

It is the income generated by the foregoing activities which allows the expansion of (C) Service Sector II that is concerned with personal and social services. The social service sector includes health care, education, community services, leisure and recreational activities. Our capacity to sustain and improve the services and opportunities depends on the capacity of sectors (A) and (B) to generate the necessary income.

The Science-Based Pyramid of Research

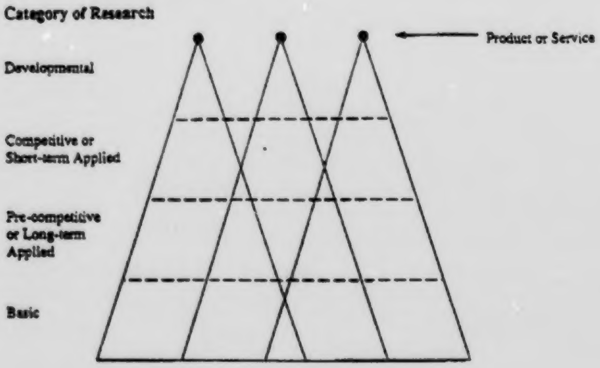

Category of Research
Product or Service
Developmental
Competitive or
Short-term Applied
Pre-competitive
or Long-term
Applied
Basic

Figure 2

Research as an element related to the overall process of innovation, can be broken down into three primary components that must be linked together to be effective:

1. Basic or fundamental research which is usually characterized by the researchers' primary objective being the generation of new knowledge and understanding about man and the world around us. This research is long-term (usually on a time-scale of 10 years or more), and has a high level of uncertainty in terms of what the results will be. In Western culture, basic research is primarily university-based and seldom results in knowledge that is of immediate commercial value. The knowledge gained from such research is rapidly and widely distributed to scientists throughout the world through publication in scientific journals. Because the results from this research are, or have been, considered a public good, this type of research has been financed primarily by the public sector and private benefactors. Increasingly, however, when knowledge contributed by basic research is critical for new product development, industries are becoming involved in basic research (OECD, 1987).

2. Applied research: In most countries, applied research is mainly carried out in industrial or government laboratories, but in some countries, there is substantial university involvement with respect to longer term research, particularly in schools of engineering, medicine and management. Applied research has a strategic target and attempts:

   - to extend the scope of understanding of materials and processes,

   - to determine how the accumulated knowledge from basic research, extend where necessary by focused specialized research, can be used to develop a potential new product or services, or

   - to determine how to modify and improve the performance of existing products or services to sustain their marketability.

   Applied research which is medium to long-term (on a time scale of three to ten years), also has a significant level of uncertainty, but because it is targeted, there is a probability that there will be economic benefit. The means for the financing of this research vary. In some sectors such as the pharmaceutical and chemical fields, the research is largely funded by the private sector primarily through the benefits from patent protection, whereas in fields such as aircraft and electronics, there has been a mixture of public and private financing. In some cases a monopoly position (e.g. AT&T and Bell Laboratories) has encouraged the funding of longer-term applied research, but there are few examples of the private sector being able to finance longer term applied research wholly from its own resources unless there is effective patent protection or the business has a monopoly position.

   It is common in some sectors to associate the processes of engineering design and development of a product or service as discussed above, with the term development or developmental research.

3. Developmental research is research that:

   - makes use of the fruits of applied research specifically to create a new marketable product or service, or

   - improves, through a series of small steps of innovation based on state-of-the-art knowledge, an already existing product or service, or

   - enhances the ease of production of a product or the provision of a service.

   This type of research has the least uncertainty, is carried out on a time scale of less than three years, and has the highest probability of economic benefit. Developmental research is mainly financed by the private sector, although there are exceptions in which there has been substantial public financing.

The foregoing categories of research can be represented by the pyramids shown in Figure 2. At the narrow peak of each pyramid is a product or service, a specific artifact of technology designed to perform a particular function in a market. From its peak each pyramid expands through the three primary categories of research to a broad base in basic research. The category "applied research" has been segmented into two slices labeled competitive (short-term applied) and pre-competitive (long-term applied). Competitive applied research is that which has direct proprietary value to the business. Pre-competitive applied research is that which is generally useful in sectors of industry (This research is often concerned with what can be called generic technology). The relative width of each slice across the pyramid suggests the range of generality of the knowledge associated with it. The overlapping of parts of the pyramids indicates that as one reaches towards the scientific roots pertinent to the development of a particular product, the knowledge base becomes relevant to a range of products. Indeed, the essence of basic science is that it seeks for general principles of understanding within particular circumstances of study, whereas engineering, through the technology it creates, seeks to realize a particular operational function in a market within the domain of possibilities bounded by science.

Science-based innovation then is innovation in which the realization of an effective and competitive product or service utilizes, through the focusing processes of the pyramid of research, the full range of scientific and engineering understanding pertinent to the function of the product or service in the marketplace.

The classification of levels of research in the research pyramid of Figure 2 is based on the diverse literature on innovation. Its pertinence for older, large-scale, science-based industries is clear. However, a key point today is that the research pyramid is relevant to all industry participating in the global economy of science-based innovation.

Copies of "INNOVATION AND CANADA'S PROSPERITY: THE TRANSFORMING POWER OF SCIENCE, ENGINEERING AND TECHNOLOGY" may be obtained by filling out the attached form.

KEYNOTE PAPER II

DERIVING BENEFITS FROM ENVIRONMENTAL RESEARCH

Stuart L. Smith, M.D.
President
RockCliffe Research and Technology Inc.
November 1988

As difficult as research can be, it is still more difficult to apply it swiftly for economic or social benefit. In addition to the usual obstacles to technology transfer, environmental research faces additional ones of a political nature. It behooves us to know a great deal more about how research is transformed into practical benefits and how environmental research in particular can be more rapidly applied. The improvement of the environment is an area where, with appropriate policies, economic and social benefits occur simultaneously.

In supporting research activities, we cannot take for granted that application will naturally follow any improvement in knowledge. More attention needs to be paid to the incentive structures of research organizations, the relationship to our industrial sector, and the interaction with political decision-making. By acting now in some specific areas, we can help guarantee that today's research will produce timely and tangible results.

SESSION B
WATER QUALITY RESEARCH
Oral Presentations

ENVIRONMENT ONTARIO PAPER

WATER QUALITY RESEARCH

Carl Schenk
Water Resources Branch
Environment Ontario

Research needs related to water and the aquatic environment cover a broad range of concerns and issues. The needs identified hereafter reflect the Ministry's interests in establishing improved treatment processes for the Province's drinking water supplies and the removal or neutralization of municipal, industrial and diffuse source wastes, developing a better understanding of cause-effect relationships in aquatic systems so that effort can be directed to deal more effectively with water quality impacts, and determining improved environmental evaluation techniques.

For convenience, water research requirements have been grouped into the following eight categories, involving 33 issues and about 130 specific needs:

1) Industrial Wastewater Treatment
2) Municipal Wastewater Treatment
3) Managing Non-Point Sources of Pollution
4) Contaminant Fate and Transport Processes in Aquatic Systems
5) Impacts of Pollutant Discharges on Aquatic Systems
6) Drinking Water
7) Effects of Acidic Deposition and Long Range Transport of Contaminants
8) Other

Research issues have been altered and the specific needs adjusted for fiscal year 1989-90 based on input from head office and regional staff throughout the Ministry. New issues include WA10 to investigate effects of intensive crop production practices on groundwater quality, WA14 to identify remedial measures to minimize the impact of lakeshore development, WA15 dealing with the aquatic effects of timber management practices, WA18 involving the development of models to address contaminant fate and transport in the Great Lakes, WA21 to model the impacts of contaminant discharges on aquatic biota, WA30 on the distribution, behaviour and effects of low level trace metals in aquatic systems, WA31 concerning the effects of organic contaminants associated with long range transport and finally, WA33 dealing with the spatial anayses of water quality.

Clearly, the Ministry's top priority in water research relates to evaluations of the significance of hazardous contaminants and minimization of these contaminants as a threat to our natural waters and drinking water supplies. This emphasis is reflected by the vast majority of the 33 research issues and related needs that have been identified. Studies carried out by universities and private sector consultants within the Ministry's

B1

BIOLOGY IN THE NEW REGULATORY FRAMEWORK FOR AQUATIC PROTECTION: MAJOR MESSAGES AND RECOMMENDATIONS FROM THE ALLISTON WORKSHOP, APRIL 26-28, 1988

K.E. Day[1], E.D. Ongley[1], R.P. Scroggins[2] and H. Eisenhauer[2]
[1]Environment Canada, Rivers Research Branch,
National Water Research Institute, Burlington, Ontario
[2]Environment Canada, Place Vincent Massey, Hull, Quebec

The new Canadian Environmental Protection Act (CEPA) together with other legislative initiatives, e.g., Ontario's Municipal Industrial Strategy for Abatement program (MISA), provides the policy framework to expand the traditional chemical approach for hazard assessment of pollutants in Canadian waters and sediments to a combination of chemical and biological toxicity tests for protection of the aquatic environment. The benefits of incorporating biological tests and standards into regulations are well recognized; however, there are reservations about the current level of scientific knowledge and practical expertise available to formulate and implement such regulations at this time.

Concerns have been expressed about the types of biological tests to be used, shifts in regulatory criteria due to improvements in the knowledge-base, the role of governments in R&D relative to the private sector and technology transfer from government laboratories to the private sector, university R&D and consulting, etc. This concern led to a national workshop, hosted by Environment Canada and chaired by the National Water Research Institute on April 26-28, 1988, at the Nottawasaga Inn, Alliston, Ontario. Called "Biology in the New Regulatory Framework for Aquatic Protection", participation included representatives from senior and technical levels of several federal government departments, provincial officials, university faculty, regulated industries, private consultants and laboratories, and municipal wastewater treatment operators. The workshop was designed to promote discussion amongst key decision makers and experts from the various sectors on the implications, problems and economic opportunities associated with incorporating biology into aquatic environmental regulations. The objective of the workshop was to provide advice to enable regulators to deal more effectively with the policy implications of biological assessment as a regulatory tool.

Invited speakers provided an overview of biological toxicity tests in Canada as they relate to CEPA, MISA and the Pest Control Products Act. Industry and the private consulting sector were invited to respond with discussions of the benefits, problems and opportunities presented by these legislations. In working groups, the participants formulated a response to the following questions: what are the benefits and limitations of biological toxicity tests as compared to other possible methods and strategies, i.e., the chemical-specific approach; what is the role of the government in research and development of biological toxicity tests; what is the role of the government in monitoring and assessment?

Full proceedings of the Alliston Workshop will be issued in a separate document by Environment Canada in 1989.

The major recommendations arising from the Workshop can be summarized as follows:

1. Governments must develop a program framework and outline policy statements required to set biology-based regulatory standards and to expedite the development of standardized protocols for biological toxicity tests.
2. Biological testing and monitoring must be integrated with chemistry in a multi-disciplinary manner when applied in hazard assessment and regulatory control.
3. Sublethal and chronic tests need to be developed to provide more sensitive monitoring tools for detecting toxic effects in ambient waters.
4. Mechanisms for improved communication, awareness and understanding of the applied uses of biological toxicity tests amongst the scientific community, regulatory agencies, industry and the public sector must be pursued.
5. Government must maintain a strong research and development capability and continue to transfer technology developed in the field of biological testing and monitoring to the private sector.
6. Government should provide guidance and show leadership in the area of biological testing and monitoring QA/QC, e.g., policies, guidelines, laboratory accreditation, toxicologist certification, etc.
7. Government is responsible for the maintenance of long-term ambient monitoring schemes while industry is responsible for the assessment of immediate areas of impact, e.g., end-of-pipe and mixing zone, with occasional auditing by the government.

B2

HYPOTHESIS TESTING IN AQUATIC TOXICOLOGY:
Basic Relationships and Simple Kinetic Modelling.

L.S. McCarty,·*; G.W. Ozburn*; A.D. Smith*, and D.G. Dixon·

* Aquatic Toxicity Research Group, Lakehead University, Thunder Bay,
· Biology Department, University of Waterloo, Waterloo

ABSTRACT

Some of the assumptions of commonly employed aquatic bioassays where toxicity or bioconcentration are estimated are reviewed. For many organic chemicals the link between bioconcentration-derived and toxicity-derived kinetics information, as well as the hypothesis that typically employed biological endpoints occur at relatively constant body burdens, can be exploited by means of a one-compartment, first-order kinetics model. When verified for the mode of toxicity and the general character of the test species, the model can be used to explore situations not explicitly examined in the original data. Such deterministic models can also be used, in the standard scientific approach of hypothesis formulation and testing, to formulate hypotheses to direct future experimental designs. Examples of applications dealing with mixtures of toxicants and intermittent exposure regimes are discussed.

INTRODUCTION

Large amounts of data concerning bioconcentration and toxicity have been collected for a variety of purposes including: monitoring, regulation development and/or support, legal requirements, and basic investigative science. Numerous chemicals, organisms and circumstances have been examined. Despite this the data available are often only of very restricted utility in new situations as the organisms or conditions or both are substantially different.

This is in large part due to the fact that aquatic toxicology has evolved largely a descriptive discipline. Studies examine the problem primarily by reporting a detailed description of the circumstances of the test and of its outcome. This stochastic approach attempts to correlate changes in results with changes in one or more experimental parameters without necessarily attempting to explain an underlying mechanism. Such descriptive methods are ultimately handicapped by the lack of an underlying general theory.

To be able to further exploit the data and increase the understanding of the phenomena aquatic toxicity studies must be directed by the traditional scientific approach: hypothesis formulation and subsequent testing. Deterministic models must be employed so that changes in results are correlated with varying experimental parameters, as in the case of the stochastic model, but also changes are also examined in terms of at least some of the fundamental biological and physical-chemical processes involved.

In this paper some of these basic concepts and assumptions will be reviewed, a examination of how simple deterministic models may be constructed from currently existing toxicity and bioconcentration data will be carried out, and hypothesis formulation and testing will be briefly explored.

Four general areas need to be examined: toxicity tests, bioconcentration (BCF) tests, toxicokinetic modelling, and the constant body toxicant concentration concept.

The primary objective of the toxicologist carrying out the toxicity bioassay is to determine the potency of the toxicant being examined relative to the potency of those which are already known (Bliss, 1957; Filov et al., 1973). There appears to be two basic explicit assumptions in this essentially stochastic approach:

1. a dose-response relationship exits, i.e. that the biological response of the organism exposed to the toxicant is some function of the amount of toxicant to which it has been exposed, and

2. the distribution of tolerance of the measured biological response of test organisms to the toxicant is known.

The assumptions require some further explanation. The dose-response relationship is the fundamental assumption of toxicology but to employ it some additional assumptions are implicit. Some measure of the amount of toxicant to which the organism is exposed, i.e. toxicant external to the organism, is being used as a surrogate for the amount of toxicant in the organism at the site(s) of toxic action which is the ultimate cause of the biological response.

Due to the complexity of non-equilibrium, non-steady-state kinetics it is usually assumed, implicitly, that a steady-state equilibrium occurs, or at least could occur, between the toxicant external to the test organisms and that which is in the body of the organism. Thus at "threshold" or "incipient" toxicity levels, with either lethal or non-lethal biological responses, it is valid to compare the potency of different toxicants (Sprague, 1969, 1970; Filov et al., 1973).

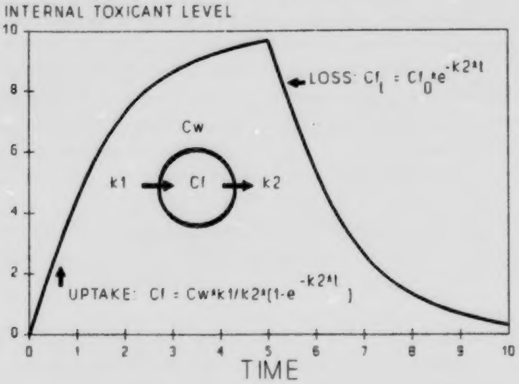
Also implicit is that the metabolism or biotransformation of the different toxicants which are being compared is similar, and usually, negligible.

The assumption of a normal distribution of the logarithm of the tolerance (log-normal) in the exposed population is commonly made as is facilitates statistical analysis (Finney, 1978).

Bioconcentration tests do not, as mentioned earlier, focus on the response aspect of the dose-response relationship, but rather emphasize the dose component. In fact, if a slightly enhanced view of the dose-response relationship - exposure, kinetic, and dynamic phases - is employed (Ariens, 1980) it is clear that most bioconcentration research is directed to the kinetics aspect - uptake, distribution, biotransformation, and elimination - of the relationship. Thus bioconcentration tests are inherently based on a dynamic model approach.

A variety of models of various levels of sophistication can be employed (Dedrick, 1986), but the common approach has been to use the simplest: the one-compartment, first-order kinetics model (1CFOK). Despite the simplicity the model has been used and discussed for a variety of chemicals and aquatic organisms (Mancini, 1983; Spacie and Hamelink, 1983; Hawker and Connell, 1985; Barber *et al.*, 1988). Figure 1 summarizes the mathematical description of this model.

Figure 1. One-Compartment, First-Order Kinetics Model: Mathematical and Graphical Form



It is becomes clear that the design and interpretation of aquatic toxicity tests is rooted in a stochastic model of the phenomena, while the basis of the bioconcentration test is a deterministic model. In previously published work (McCarty, 1987a,b) it has been suggested that, at least for poorly metabolized, neutral, narcotic organic chemicals, information from both toxicity tests and bioconcentration tests could be integrated since, essentially, each was focused on an opposite side of the same coin. That is to say, the kinetics should be similar in both cases and differences that occur should primarily be a result of the difference of the endpoints being employed. Since the biological response is a function of the amount of toxicant which enters the body of the organism both bioassay endpoints will be referenced to a common basis, the whole body burden of toxicant.

The whole-body concentration or burden - an internal level - is an approximation of the toxic dose which is more closely associated with the biological response than the concentration or dose of toxicant which is applied externally to the test organisms. The toxicant bound to the receptors at the site of toxic action is a much more accurate measure, although considerably more difficult to estimate. Thus the body burden or concentration, incorporating adjustments for known influencing factors such as varying lipid levels, will be used as a reasonable first approximation estimate of the actual effective internal dose.

Concentration, either internal or external, is itself is an approximation for the effective dose in the organism since, as elucidated by Ferguson (1939), it is the activity of the number of molecules of toxicant in the organism and not their actual number which is thought to be most closely related to the biological response.

Therefore, in toxicity bioassays, within a common mode of toxic action and species and condition of test organism, the body burden of toxicant associated with the toxic endpoint being employed should be relatively constant (Connolly, 1985). Support for this comes from QSAR work. McCarty (1986, 1987a), working with toxicity and bioconcentration data, reported that for the acute toxicity of some neutral, narcotic, chlorinated hydrocarbons the body burden associated with 96 h LC50 estimates appeared to be about 2 mmole/L internal concentration for fish of about 5% lipid content (2 mmole/kg if fish density is 1.0).

More recently van Hoogen and Opperhuizen (1988) actually measured body burdens of chlorobenzenes at the 96 h LC50 and found a body burden estimate of about 2.5 mmole/L in fish with a 5% lipid level, which is in remarkably good agreement with the predicted value.

It must be noted that, in the typical toxicity test, the toxicity body burden is being measured indirectly, by the mortality response of the exposed population, and this and other variability associated with the estimation of tolerance distribution by this means will be incorporated body burdens estimated in this way.

For the bioconcentration bioassay the body burden is actually measured in the test so estimates should be more accurate. For many commonly studied organic chemicals it appears that the ratio of the exposure concentration to the body burden is relatively constant over a range of exposure concentrations and follows the well-established relationship of increasing body burden at steady-state equilibrium as a function of the lipophilicity of the chemical (Mackay, 1982).

It becomes clear that the examination of the relationships between data derived from standard toxicity and bioconcentration bioassays requires accepting several assumptions. Rather than detail these assumptions further we will simply state the assumptions that are the basis for the working hypothesis.

1. The uptake and elimination of many organic chemicals, especially neutral narcotics, can be reasonably approximated by a one-compartment, first-order kinetics model which is independent of the biological activity or response endpoint under examination,

2. for the case of neutral, narcotic organics with the same mode of toxic action the biological response endpoints estimated by the statistical response of the exposed population, and commonly employed in aquatic bioassays: lethality (eg. threshold LC50), growth, and inhibition of reproduction, are a function of a relatively constant body burden of the toxicant.

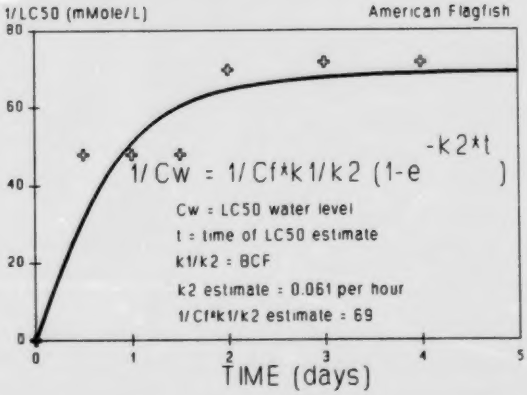
As well constant bioavailability of the toxicant and no uptake of toxicant from the diet, negligible biodegradation and growth for the duration of exposure is assumed.

METHODOLOGY AND RESULTS

Toxicity information for 1,4-dichlorobenzene were obtained in continuous-flow, acute toxicity tests with juvenile American flagfish (*Jordanella floridae* Goode and Bean) of an age of 2 to 4 months (Smith *et al.*, manuscript). The estimated 96 h LC50 was 2.05 mg/L (14.0 µmole/L). Samples from a series of tests indicated fish sizes ranged from 0.3 to 5 g (typically 0.5 to 4.0 g) and lipid contents varied from 7 to 16 per cent (typically 8 to 12 %). A non-toxic (less than 2 % of the LC50) level of acetone was used as a solubilizing agent.

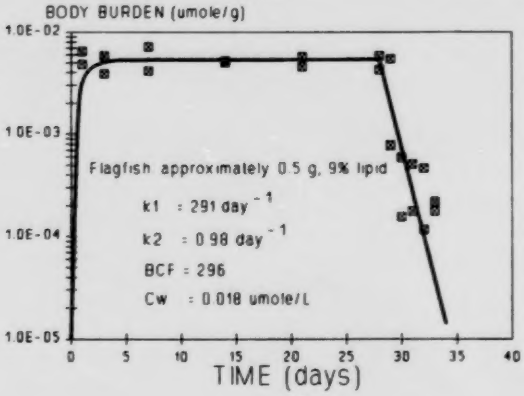
The time-toxicity data were used to obtain estimates of K2, the elimination rate, and the factor 1/Cf*K1/K2, which is the product of the inverse of the fish toxicant concentration times the bioconcentration as discussed by McCarty (1987b). The nonlinear routine from the PC-based statistics program SYSTAT 4.0 was employed. Figure 2 summarizes the approach and the results.

Figure 2. Non-Linear Curve Fit of Acute Toxicity of 1,4-Dichlorobenzene at Various Times



The bioconcentration data were obtained with fish of similar characteristics employed in the toxicity testing. The fish were exposed for 28 days to a measured concentration of 2.7 µg/L (0.018 µmole/L) of 1,4-DCB in 79 mg/L acetone followed by a 14 day depuration period. (ATRG, 1987). The exposure water concentrations and body burden estimates at different times were analyzed with the BIOFAC program to generate kinetics constants and a bioconcentration factor based on the whole body toxicant level. The BIOFAC program is essentially a nonlinear curve-fitting routine customized for use in analysis of bioconcentration data (Blau and Agin, 1978). The data and results are presented in Figure 3.

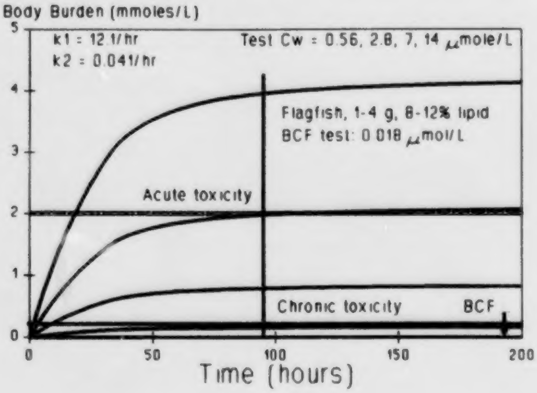
Figure 3. 1,4-Dichlorobenzene Bioconcentration Kinetics: BIOFAC Model Analysis



Hypothesis Formulation

Since our first assumption is that kinetics are independent of the biological response being investigated it follows that, allowing for variance due to experimental factors and organism sensitivity, the kinetics from a bioconcentration test should be very similar to those obtained in a toxicity test. Thus we have taken the kinetics information from Figure 3 and modeled the time course of toxicant concentrations in the body which should occur when flagfish are exposed to various water levels of 1,4-DCB as indicated in Figure 4. Also indicated is the approximate body burden associated with the 96 h LC50, 2 mmole/L.

Figure 4. Hypothesis: Uptake of 1,4-Dichlorobenzene at Various Water Concentrations



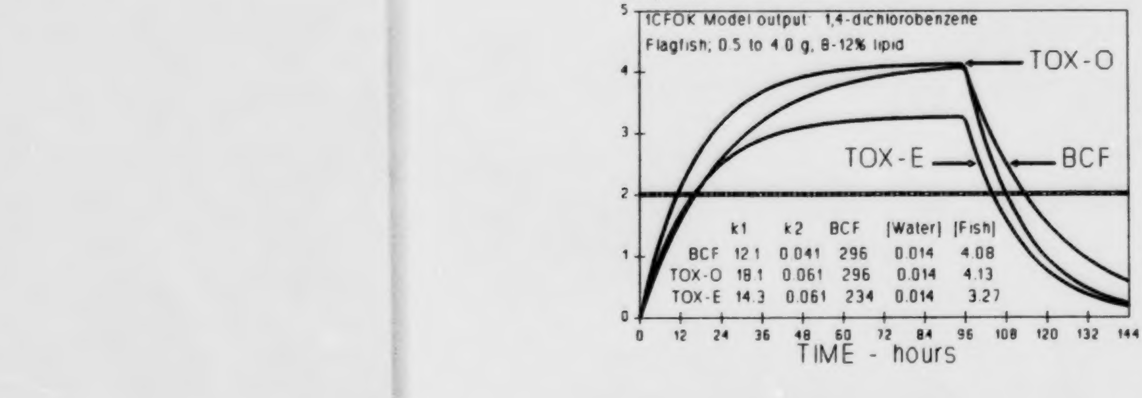
Test of the Hypothesis

In Figure 4 the time-course of body burden accumulation has been modeled for several endpoints; however, the most practical endpoint for use in testing the hypothesis is the threshold LC50 endpoint as it is currently the most well-defined . Although the body-burden estimate associated with this endpoint would more realistically represented by a confidence interval about the mean estimate, further refinement is currently being carried out and this value is all that is currently available.

Figure 5 is the one-compartment, first-order kinetics model output for a 96 h exposure to a concentration of 0.014 mmole/L of 1,4-dichlorobenzene, the estimated 96 h LC50 for these organisms. The model was run under three sets of parameters, identified as BCF, TOX-O, and TOX-E, and the results are presented in the figure.

The model output labelled BCF is based on information - k1, k2, and bioconcentration factor (BCF) - taken entirely from the bioconcentration bioassay data.

Figure 5. Hypothesis Test: Uptake of 1,4-Dichlorobenzene at Estimated LC50 Water Concentration



	k1	k2	BCF	[Water]	[Fish]
BCF	12.1	0.041	296	0.014	4.08
TOX-O	18.1	0.061	296	0.014	4.13
TOX-E	14.3	0.061	234	0.014	3.27

In the observed bioconcentration-toxicity (TOX-O) output the elimination constant, k2, was derived from the non-linear curve fitting of the time-acute toxicity data presented in Figure 2 while the BCF value is from the bioconcentration results. The uptake constant k1 is derived from these two values.

For the case of the estimated toxicity (TOX-E) output the k2 estimate is from the toxicity curve-fitting data while the BCF parameter estimate is calculated from Mackay's (1982) relationship for bioconcentration and Kow. A log Kow estimate for 1,4-dichlorobenzene of 3.52, a measured estimate from the MED CHEM 3.53 database (Leo, 1988), was used in this calculation. The constant in the Mackay's equation was changed from 0.05 to 0.10 to reflect the higher lipid content of the flagfish used in this study versus. Fish lipid levels were likely closer to 5 % in the data used by Mackay (1982) while, as noted earlier, 10 % is a more reasonable approximation for the flagfish used in this study.

From inspection of the figure it can be seen that all three of the curves plotted are in very close proximity to each other. In addition the estimated toxicant concentration in the fish are close and compare very favourably with the estimates of toxicant body burden estimated for acute toxicity in other studies - 2 and 2.5 mmole/L - noted earlier.

DISCUSSION

Although this study is not an exhaustive evaluation of the problem it would seem reasonable, given the close similarity of the different uptake curves and the agreement of the model-estimated toxic body burdens with an independent estimate of body burden for the same toxic endpoint, to conclude that the hypothesis which was proposed has been validated for the circumstances considered. Thus it appears that:

1. Kinetics information from toxicity and bioconcentration tests is very similar, and

2. a one-compartment, first-order kinetics model appears to be a the deterministic model which, at least in the first approximation, is adequate for the study of toxicant kinetics and dynamics in aquatic bioassays measuring both acute toxicity and bioconcentration.

These conclusions are limited to the circumstances which were examined; however, since the deterministic model approach has been validated it can be employed as the basis for the investigative procedure of hypothesis formulation and testing.

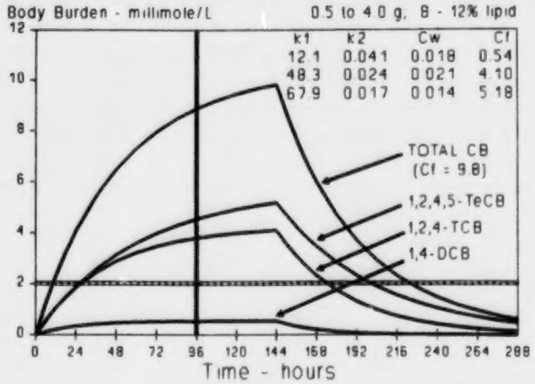
Two important areas can be addressed in this way. First, currently available bioassay results can be better interpreted and interpolated while results for new chemicals and/or circumstances can be predicted. Second, the toxicological significance of body burdens or time series of changing body burdens of organisms collected in the field can be estimated and compared with data obtained under more rigorously controlled situations.

An example of an application of the hypothesis formulation and testing procedure applied in the first area discussed above appears in Figure 6. The k1 and k2 values for 1,2,4-trichlorobenzene and 1,2,4,5-tetrachlorobenzene were obtained from bioconcentration tests carried out exactly as described earlier for 1,4-dichlorobenzene, which is also included in this figure. The model output indicates hypothesized body toxicant changes at a exposure concentrations ten times that employed in the original bioconcentration bioassays. In addition the sum of the body burdens of the three chlorobenzenes is obtained at each sampling time and plotted as the total chlorobenzene body burden.

Experiments could be designed and carried out to test several aspects of this hypothesis. Bioconcentration bioassays could be carried out at water concentrations ten times the original water concentrations to examine success of the model predictions for the time course and ultimate body burden for each of the three chlorobenzenes. As well a study of the bioaccumulation of a mixture of the three chlorobenzenes would determine the success of the model in prediction the bioaccumulation of a mixture.

In addition, since body burden levels at the higher exposure levels used in the model output are of the order of magnitude of that associated with substantial acute lethality, toxicity tests could be

Figure 6. Model Output for a Mixture of 3 Chlorobenzenes

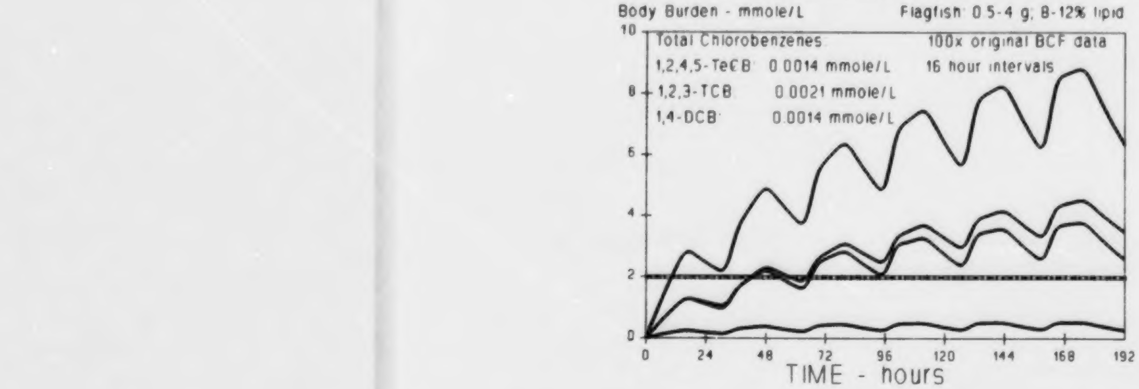


carried out, both with single chemicals and with a mixture, to determine the relative toxicities.

If the model reasonably describes the circumstances a difference in the mortality caused by various chlorobenzenes should be apparent. For example, population mortality of say 20% for 1,4-DCB and 50% for either 1,2,4-TCB or 1,2,4,5-TeCB (these latter two are likely so close as be distinguishable under the test conditions) might be expected, or at least differences in mortalities in this order. If the toxicity of the three CBs are simply additive then a toxicity test employing a mixture of the three would be expected to produce a higher percent mortality, say 80%, than the single chemical exposures and the onset of mortality might be expected to be earlier than the others, as suggested by the shape of the Total CB output curve in Figure 6.

Another aspect which could be investigated is the situation where the exposure to the toxicant is not continuous i.e. intermittent or pulse exposures (Holdway and Dixon, 1985). Since a kinetics-based dynamic model is being employed it is a relatively simple mathematical exercise to predict the body burden through various changing exposure levels. The test of the hypothesis is an actual experiment to see if the biological response reflects the responses expected and/or if the body burdens follow the predicted course. Figure 7 illustrates the hypothesized body burdens for a situation similar to that shown in Figure 5 but with the exposure being of an intermittent or pulse nature, 16 hours of exposure to toxicant followed by 16 hours of exposure to clean water for a total of 6 cycles. Experiments could be designed to examine body burdens and toxicity of the 3 chlorobenzenes, both individually and in mixtures, in a manner similar to the continuous exposure investigations.

Figure 7. Model Output: A Mixture of 3 Chlorobenzenes with a Intermittent Exposure Regime



Experimental data examining individual chemical toxicity, mixture toxicity and bioconcentration, and intermittent exposure toxicity is currently being collected and/or analyzed in our laboratories and we hope to report on these activities in the near future.

We trust that, within the limitations specified, we have demonstrated how toxicity and bioconcentration bioassay data are related and how this information might be exploited within the confines of a deterministic model based on the concepts of one-compartment, first-order kinetics and the association of a biological response with a relatively fixed body toxicant burden. Furthermore, we trust that we have illustrated how this approach can be used to in hypothesis formulation and testing, both in interpolating and extrapolating existing data as well as in experimental design.

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B3

Effects of mixed metal mining waste on white sucker populations: development of a framework to describe fish population responses to environmental change.

K.R. Munkittrick[1] and D.G. Dixon. Dept. of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1 ([1]current address: Dept. Zoology, University of Guelph, Guelph, Ontario N1G 2W1)

Objectives

The project was undertaken in 1985 as an integrated field-laboratory program designed to determine the impacts of copper and zinc contamination on white sucker (*Catostomus commersoni*) populations in several lakes in the Manitouwadge district of Northern Ontario. The Manitouwadge lakes were selected because the levels of copper (13 to 15 ug/l) and zinc (209 to 253 ug/l) were slightly higher than Canadian water quality guidelines. The water quality guideline approach is based on the assumption that lakes of similar characteristics exposed to similar concentrations of metals will exhibit similar effects. A previous study conducted in the mid-1970s had documented changes in white sucker populations exposed to levels of copper (12 ug/l) and zinc (245 ug/l) (McFarlane and Franzin 1978) close to those identified at the Manitouwadge site. To test the impacts of metals, and the water quality guideline approach, we set out to examine the white sucker populations in the Manitouwadge district.

Our original objectives were to 1) examine the growth and reproduction of white sucker populations in the Manitouwadge chain, 2) to examine the larvae originating from the contaminated sites for evidence of acclimatory changes and 3) to develop a protocol for identifying the impacts of chemical contamination on fish populations. Samples were collected from six lakes in the Manitouwadge chain over the period of 1985-1987. White sucker were evaluated for evidence of impact on growth, reproductive performance, larval survival and tolerance of larvae to copper and zinc exposure.

Summary

White sucker reached the age of maturity between 4 and 6 years of age at all sites, and until 6 years of age there were no differences in length or weight of fish from control and Manitouwadge (contaminated) sites (Munkittrick and Dixon 1988a). After maturity, fish from contaminated sites were significantly smaller and shorter than those from control sites. In addition, fish from contaminated lakes also exhibited decreases in fecundity and egg size, failed to show significant increases of fecundity with age and exhibited an increased incidence of spawning failure (Munkittrick and Dixon 1988a,b).

Examination of the reproductive performance did not detect differences between white sucker collected at contaminated and control sites (Munkittrick and Dixon 1988b). There were also no differences in the fertilization rates of naturally-fertilized eggs and in cross-fertilization trials the metal-exposed males performed better than control males in fertilization trials with control eggs.

Larvae hatched from eggs collected at contaminated sites were smaller, developed at a slightly increased rate, and exhibited poorer growth and survival than larvae from control sites (Munkittrick and Dixon 1988b). These changes were evident despite the fact that the contaminated eggs were fertilized and hatched in clean water, and the differences are consistent with both the phenomena of decreased female reproductive commitment and the vertical transmission (mother to offspring) of contaminant residues.

The failure of female white sucker to grow significantly after maturity, and the decreased energetic commitment to reproduction suggested that the food base in the contaminated lakes was limiting the performance of the female white sucker. Female fish exhibited decreased muscle lipid levels, decreased serum lipid levels during the postspawning period and an apparent decrease in visceral lipids during the autumn. There were no effects of collection site on body stores of liver glycogen, liver lipids, serum triglycerides, total serum cholesterol (Munkittrick and Dixon 1988a) or brain amine levels (Munkittrick et al. 1988a).

Larvae showed significant changes in tolerance and resistance to copper and zinc with age and metal resistance peaked at the time of the onset of liver functioning. Larvae from contaminated eggs showed increased resistance and tolerance to waterborne copper during the periods of endogenous nutrition, despite the fact that the eggs were not pre-exposed to exogenous metals (Munkittrick and Dixon 1988b). The effect was not seen in larvae at first feeding, at ages older than 4 d after the onset of feeding, suggesting that the change was not genetic in origin.

The effects was also absent in larvae hatched from control eggs fertilized with sperm taken from males at contaminated sites. Fertilization of eggs collected at the contaminated site with milt collected from control males yielded larvae whose tolerance and resistance profiles could not be distinguished from the Manitouwadge larvae (Munkittrick and Dixon 1988c). The fact that the increase in tolerance was associated with eggs from the contaminated site and not the milt suggests the presence of a maternal yolk factor associated with increased resistance and tolerance of larvae to copper. The factor appears to be metal residues transferred in the yolk, and no differences were detected in egg metallothionein residues between control and contaminated sites (Munkittrick and Dixon 1988c).

Eggs from the Manitouwadge site were significantly smaller than control eggs, and naturally-fertilized eggs collected from the contaminated spawning sites exhibited a further decrease in egg size and an increase in deformity rate not evident in contaminated eggs manually fertilized in control water (Munkittrick and Dixon 1988c). Incubation trials involving the placement of eggs in streams flowing out of the tailings area resulted in a decreased egg size and tolerance to copper and an increased deformity rate (Munkittrick and Dixon 1988c). Both changes were associated with the influx of metals during the water-hardening process.

The distribution of metals in white sucker tissues was monitored, and elevations in both copper and zinc residues were identified in liver, kidney, gill and gonadal tissue (Miller et al. 1988). Muscle levels of zinc were actually significantly lower at contaminated sites than at controls. There was evidence of metal uptake from the diet and the concentrations of metals in gut contents exceeded 400 ppm Cu and 1200 ppm Zn. Analysis of sediment metal concentrations showed elevations in both Cu and Zn at contaminated sites (Miller et al. 1988; Munkittrick et al. 1988b).

Additional work shows that several major food groups are missing from the sediments of contaminated sites (Munkittrick et al. 1988b), and previous work suggests that sediments under water deeper than 5 m may be incapable of supporting macroinvertebrate fauna (German 1971; Pugh and Maki 1986). Analysis of benthic samples collected from near-shore areas (< 3 m depth) at the contaminated sites indicated a decreased abundance or absence of pollution-sensitive groups such as ephemeroptera, plecoptera, odonata, hirudinea, unionid clams, gastropods, amphipods and aquatic beetles (Munkittrick et al. 1988). Fauna at the contaminated sites was dominated by chironomids and other dipterans.

White sucker stomach contents showed marked differences between the sites (Munkittrick et al. 1988b; Munkittrick and Dixon 1988d). Fish collected at the control sites had an average of 7.8 organisms per stomach and were dominated by ephemeropteran larvae. Stomach samples collected at Manitouwadge yielded an average of 49.8 organisms per stomach and were dominated by chironomid larvae (29 per stomach) (Munkittrick et al. 1988b; Munkittrick and Dixon 1988d). The absence of ephemeroptean larvae at contaminated sites and a decreased chironomid density in the sediments would result in a marked decrease in feeding efficiency at the contaminated sites.

Changes in biochemical parameters indicative of chronic stress were non-existant on inconsistent (Munkittrick and Dixon 1988a; Munkittrick et al. 1988a). Effects on the growth, fecundity and lipid status of white sucker could be attributed to nutritional deficiencies related to the decreased food abundance and density at contaminated sites, which could be related to the increased sediment metal levels. Sediment metal burdens have declined substantially since the late 1960's (German 1971). Direct effects of the metals were detected on the larvae hatching from eggs collected at contaminated sites. Evidence for direct effects on egg size and larval deformities were related to increased metal burdens in the eggs. This increase could be related to both the entry of metals during the water-hardening process at contaminated sites and the vertical transmission of metal residues from the female through the yolk.

In summary, white sucker collected from the Manitouwadge site showed a decreased growth rate and fecundity, with no apparent changes in mean age, condition factor or egg fertilization ability. This does not compare well with McFarlane and Franzin's (1978) findings of increased growth rate and fecundity, decreased mean age and decreased reproductive performance. This is surprising since both lakes were exposed to similar waterborne metal concentrations, and were of similar size and water hardness. The apparent inability to generalize ecosystem responses to seemingly identical chemical stressors complicates our abilities to predict ecosystem responses without detailed field sampling.

There is a need to develop simple and inexpensive methods to follow fish population responses to environmental degradation or lake restoration. In addition to field and laboratory testing, a framework was developed as a simple, cost-effective, rapid mechanism for assessment of toxicant impact on aquatic environments (Munkittrick and Dixon 1988d,e,f). The framework, Population Indicators of Sublethal Contaminant Effects on Suckers (PISCES), separates response patterns based on population characteristics. The framework is an adaptation of Colby's (1984) descriptions of fisheries exploitation impacts on fish populations.

The status of fish populations is a reflection of the overall condition of the aquatic environment. The framework assumes that changes in the death or birth rates of fish populations, or alterations in the availability of food or habitat are associated with characteristic responses of sucker populations (MUnkittrick and Dixon 1988e). The responses have been grouped into five main patterns based on population characteristics such as mean age, fecundity and condition factor. The patterns correspond to direct effects on adults (exploitation), recruitment failure, multiple stressors, food limitation and niche shifts. Populations which are growing, reproducing or surviving at rates which are indistinguishable from a reference (control) population are considered free from adverse chemical effects.

The application of PISCES to this study, and to several previously published data sets, showed that white sucker populations responded to environmental stressors in predictable patterns (Munkittrick and Dixon 1988e). The system can also be applied to populations of other species of fish, including salmonids, percids and centrarchids (Munkittrick and Dixon 1988f). However, there are limitations associated with the selection of sentinel species and sampling sites which must be taken into consideration (Munkittrick and Dixon 1988e).

The use of the framework is limited by the selection and appropriate sampling of a comparable control population (Munkittrick and Dixon 1988e). Additional limitations include the lack of dose-response sensitivity and predictive ability. In spite of the limitations, the PISCES approach does offer researchers looking at field sites an early indication of the site of stressor impact on an ecosystem and can provide useful information for the design of sampling schedules and derivation of useful, testable hypotheses. When changes are not correctly predicted, the use of contrasting, generalized response patterns can act to direct and focus research efforts on crucial areas impacted by changing conditions. The availability of historical data sets and information from angler or fisheries harvests allows the PISCES system to be easily adapted for monitoring purposes (Munkittrick and Dixon 1988e).

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Effects of mixed metal mining waste on white sucker populations: development of a framework to describe fish population responses to environmental change.

K.R. Munkittrick[1] and D.G. Dixon. Dept. of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1 ([1]current address: Dept. Zoology, University of Guelph, Guelph, Ontario N1G 2W1)

Objectives

The project was undertaken in 1985 as an integrated field-laboratory program designed to determine the impacts of copper and zinc contamination on white sucker (*Catostomus commersoni*) populations in several lakes in the Manitouwadge district of Northern Ontario. The Manitouwadge lakes were selected because the levels of copper (13 to 15 ug/l) and zinc (209 to 253 ug/l) were slightly higher than Canadian water quality guidelines. The water quality guideline approach is based on the assumption that lakes of similar characteristics exposed to similar concentrations of metals will exhibit similar effects. A previous study conducted in the mid-1970s had documented changes in white sucker populations exposed to levels of copper (12 ug/l) and zinc (245 ug/l) (McFarlane and Franzin 1978) close to those identified at the Manitouwadge site. To test the impacts of metals, and the water quality guideline approach, we set out to examine the white sucker populations in the Manitouwadge district.

Our original objectives were to 1) examine the growth and reproduction of white sucker populations in the Manitouwadge chain, 2) to examine the larvae originating from the contaminated sites for evidence of acclimatory changes and 3) to develop a protocol for identifying the impacts of chemical contamination on fish populations. Samples were collected from six lakes in the Manitouwadge chain over the period of 1985-1987. White sucker were evaluated for evidence of impact on growth, reproductive performance, larval survival and tolerance of larvae to copper and zinc exposure.

Summary

White sucker reached the age of maturity between 4 and 6 years of age at all sites, and until 6 years of age there were no differences in length or weight of fish from control and Manitouwadge (contaminated) sites (Munkittrick and Dixon 1988a). After maturity, fish from contaminated sites were significantly smaller and shorter than those from control sites. In addition, fish from contaminated lakes also exhibited decreases in fecundity and egg size, failed to show significant increases of fecundity with age and exhibited an increased incidence of spawning failure (Munkittrick and Dixon 1988a,b).

Examination of the reproductive performance did not detect differences between white sucker collected at contaminated and control sites (Munkittrick and Dixon 1988b). There were also no differences in the fertilization rates of naturally-fertilized eggs and in cross-fertilization trials the metal-exposed males performed better than control males in fertilization trials with control eggs.

Larvae hatched from eggs collected at contaminated sites were smaller, developed at a slightly increased rate, and exhibited poorer growth and survival than larvae from control sites (Munkittrick and Dixon 1988b). These changes were evident despite the fact that the contaminated eggs were fertilized and hatched in clean water, and the differences are consistent with both the phenomena of decreased female reproductive commitment and the vertical transmission (mother to offspring) of contaminant residues.

The failure of female white sucker to grow significantly after maturity, and the decreased energetic commitment to reproduction suggested that the food base in the contaminated lakes was limiting the performance of the female white sucker. Female fish exhibited decreased muscle lipid levels, decreased serum lipid levels during the postspawning period and an apparent decrease in visceral lipids during the autumn. There were no effects of collection site on body stores of liver glycogen, liver lipids, serum triglycerides, total serum cholesterol (Munkittrick and Dixon 1988a) or brain amine levels (Munkittrick et al. 1988a).

Larvae showed significant changes in tolerance and resistance to copper and zinc with age and metal resistance peaked at the time of the onset of liver functioning. Larvae from contaminated eggs showed increased resistance and tolerance to waterborne copper during the periods of endogenous nutrition, despite the fact that the eggs were not pre-exposed to exogenous metals (Munkittrick and Dixon 1988b). The effect was not seen in larvae at first feeding, at ages older than 4 d after the onset of feeding, suggesting that the change was not genetic in origin.

The effects was also absent in larvae hatched from control eggs fertilized with sperm taken from males at contaminated sites. Fertilization of eggs collected at the contaminated site with milt collected from control males yielded larvae whose tolerance and resistance profiles could not be distinguished from the Manitouwadge larvae (Munkittrick and Dixon 1988c). The fact that the increase in tolerance was associated with eggs from the contaminated site and not the milt suggests the presence of a maternal yolk factor associated with increased resistance and tolerance of larvae to copper. The factor appears to be metal residues transferred in the yolk, and no differences were detected in egg metallothionein residues between control and contaminated sites (Munkittrick and Dixon 1988c).

Eggs from the Manitouwadge site were significantly smaller than control eggs, and naturally-fertilized eggs collected from the contaminated spawning sites exhibited a further decrease in egg size and an increase in deformity rate not evident in contaminated eggs manually fertilized in control water (Munkittrick and Dixon 1988c). Incubation trials involving the placement of eggs in streams flowing out of the tailings area resulted in a decreased egg size and tolerance to copper and an increased deformity rate (Munkittrick and Dixon 1988c). Both changes were associated with the influx of metals during the water-hardening process.

The distribution of metals in white sucker tissues was monitored, and elevations in both copper and zinc residues were identified in liver, kidney, gill and gonadal tissue (Miller et al. 1988). Muscle levels of zinc were actually significantly lower at contaminated sites than at controls. There was evidence of metal uptake from the diet and the concentrations of metals in gut contents exceeded 400 ppm Cu and 1200 ppm Zn. Analysis of sediment metal concentrations showed elevations in both Cu and Zn at contaminated sites (Miller et al. 1988; Munkittrick et al. 1988b).

Additional work shows that several major food groups are missing from the sediments of contaminated sites (Munkittrick et al. 1988b), and previous work suggests that sediments under water deeper than 5 m may be incapable of supporting macroinvertebrate fauna (German 1971; Pugh and Maki 1986). Analysis of benthic samples collected from near-shore areas (< 3 m depth) at the contaminated sites indicated a decreased abundance or absence of pollution-sensitive groups such as ephemeroptera, plecoptera, odonata, hirudinea, unionid clams, gastropods, amphipods and aquatic beetles (Munkittrick et al. 1988). Fauna at the contaminated sites was dominated by chironomids and other dipterans.

White sucker stomach contents showed marked differences between the sites (Munkittrick et al. 1988b; Munkittrick and Dixon 1988d). Fish collected at the control sites had an average of 7.8 organisms per stomach and were dominated by ephemeropteran larvae. Stomach samples collected at Manitouwadge yielded an average of 49.8 organisms per stomach and were dominated by chironomid larvae (29 per stomach) (Munkittrick et al. 1988b; Munkittrick and Dixon 1988d). The absence of ephemeroptean larvae at contaminated sites and a decreased chironomid density in the sediments would result in a marked decrease in feeding efficiency at the contaminated sites.

Changes in biochemical parameters indicative of chronic stress were non-existant on inconsistent (Munkittrick and Dixon 1988a; Munkittrick et al. 1988a). Effects on the growth, fecundity and lipid status of white sucker could be attributed to nutritional deficiencies related to the decreased food abundance and density at contaminated sites, which could be related to the increased sediment metal levels. Sediment metal burdens have declined substantially since the late 1960's (German 1971). Direct effects of the metals were detected on the larvae hatching from eggs collected at contaminated sites. Evidence for direct effects on egg size and larval deformities were related to increased metal burdens in the eggs. This increase could be related to both the entry of metals during the water-hardening process at contaminated sites and the vertical transmission of metal residues from the female through the yolk.

In summary, white sucker collected from the Manitouwadge site showed a decreased growth rate and fecundity, with no apparent changes in mean age, condition factor or egg fertilization ability. This does not compare well with McFarlane and Franzin's (1978) findings of increased growth rate and fecundity, decreased mean age and decreased reproductive performance. This is surprising since both lakes were exposed to similar waterborne metal concentrations, and were of similar size and water hardness. The apparent inability to generalize ecosystem responses to seemingly identical chemical stressors complicates our abilities to predict ecosystem responses without detailed field sampling.

There is a need to develop simple and inexpensive methods to follow fish population responses to environmental degradation or lake restoration. In addition to field and laboratory testing, a framework was developed as a simple, cost-effective, rapid mechanism for assessment of toxicant impact on aquatic environments (Munkittrick and Dixon 1988d,e,f). The framework, Population Indicators of Sublethal Contaminant Effects on Suckers (PISCES), separates response patterns based on population characteristics. The framework is an adaptation of Colby's (1984) descriptions of fisheries exploitation impacts on fish populations.

The status of fish populations is a reflection of the overall condition of the aquatic environment. The framework assumes that changes in the death or birth rates of fish populations, or alterations in the availability of food or habitat are associated with characteristic responses of sucker populations (MUnkittrick and Dixon 1988e). The responses have been grouped into five main patterns based on population characteristics such as mean age, fecundity and condition factor. The patterns correspond to direct effects on adults (exploitation), recruitment failure, multiple stressors, food limitation and niche shifts. Populations which are growing, reproducing or surviving at rates which are indistinguishable from a reference (control) population are considered free from adverse chemical effects.

The application of PISCES to this study, and to several previously published data sets, showed that white sucker populations responded to environmental stressors in predictable patterns (Munkittrick and Dixon 1988e). The system can also be applied to populations of other species of fish, including salmonids, percids and centrarchids (Munkittrick and Dixon 1988f). However, there are limitations associated with the selection of sentinel species and sampling sites which must be taken into consideration (Munkittrick and Dixon 1988e).

The use of the framework is limited by the selection and appropriate sampling of a comparable control population (Munkittrick and Dixon 1988e). Additional limitations include the lack of dose-response sensitivity and predictive ability. In spite of the limitations, the PISCES approach does offer researchers looking at field sites an early indication of the site of stressor impact on an ecosystem and can provide useful information for the design of sampling schedules and derivation of useful, testable hypotheses. When changes are not correctly predicted, the use of contrasting, generalized response patterns can act to direct and focus research efforts on crucial areas impacted by changing conditions. The availability of historical data sets and information from angler or fisheries harvests allows the PISCES system to be easily adapted for monitoring purposes (Munkittrick and Dixon 1988e).

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B4

AN EXAMINATION OF THE CHRONIC TOXICITY OF THIOCYANATE TO FRESHWATER FISH FOR THE DEVELOPMENT OF A WATER QUALITY CRITERION,

R.P. Lanno and D.G. Dixon, Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1.

Introduction

Cyanide (CN^-) is used by the mining industry in the extraction and concentration of gold and silver from their respective ores. Both froth flotation and leaching utilize CN^- for solubilization and complexation. As a result cyanides are routinely present in mine effluents in considerable quantities. This situation has long been recognized as an environmental problem and has resulted in the establishment of an Ontario water quality objective for CN^- (0.005 mg/L, as HCN) (Ontario Ministry of the Environment, 1984).

A number of processes have been developed for the elimination of CN^- from mine effluents. Cyanide is often complexed with sulphur, either from sulphur dioxide or an inorganic polysulphide, to form thiocyanate (SCN^-). Although SCN^- appears to be much less toxic than CN^-, there is relatively little scientific evidence to fully substantiate this observation. As a result, there is currently no water quality objective for SCN^- in Ontario and no sound data base to establish one.

The 96-h LC50 values for SCN^- for freshwater fish range from 50 to 230 mg/L (Speyer and Raymond, 1985; Doudoroff, 1976), suggesting that SCN^- is substantially less toxic than HCN. Acute toxicity data for SCN^- provides no information on the long-term effects of SCN^- on the growth and reproduction of freshwater fish. Also, the toxic mode of action of SCN^- has not been identified, but often leads to a sudden, violent death termed Sudden Death Syndrome (SDS) by Heming et al. (1985).

The objectives of our research are to obtain sufficient data on the long-term sublethal toxicity of SCN^- on the growth, metabolism and reproduction of freshwater fish to permit the establishment of a water quality criterion. An attempt will also be made to apply laboratory results to a field situation in the gold mining region of northern Ontario.

Materials and methods

Laboratory studies

The laboratory portion of the study has been divided into two phases: 1) The long-term sublethal exposure of rainbow trout fry to SCN^- to determine effects on growth and metabolism, and to characterize a syndrome of sublethal SCN^- toxicity. 2) A life-cycle study on the effects of sublethal exposure to SCN^- on the reproductive capacity of fathead minnows.

The growth trial portion of the rainbow trout phase of the study has been completed and analysis of data is currently underway. The fathead minnow portion of the study has just recently been started.

Acute baseline bioassays

Rainbow trout (2 g) were exposed to various concentrations of SCN^- for a 96 h period. Mortalities were recorded at various time intervals up to 96 h. At 96 h, fish were stressed by a 15 s pursuit with a hand held dip net, and subsequent mortalities were recorded 30 minutes after the application of the stressor.

Chronic exposure studies

Triplicate groups of rainbow trout (2 g) were continuously exposed to nominal SCN^- concentrations of 40, 80, 120 and 160 mg/L for 16 weeks. Fish were randomly allotted to 60 L, white fibreglass tanks and fish weights were standardized to a coefficient of variation of <3% the day before the commencement of SCN^- administration. Trout were pair-fed a practical trout diet (GRT-70) (Cho et al., 1974). Each treatment tank was randomly matched with a control tank within its block. Treatment tanks were fed ad libitum and the ration weighed, four times per day. An equal weight of food was then fed to the matched control tank for each treatment replicate. Trout were weighed every two weeks and the weighing procedure was also used as a routine stressor to measure the expression of SDS within each treatment population. Feed intake, mortalities and feeding behaviour were monitored daily.

At the termination of the 16 week growth study, fish were anaesthetized in MS222 and killed by cervical dislocation. Length, weight, and splenosomatic and hepatosomatic indices were measured. Hematocrit and hemoglobin determinations were performed. Plasma samples for the colourimetric determination of total plasma cyanide-reactive substances (Lambert et al., 1975) and plasma thyroxine (T_4) and tri-iodothyronine (T_3) were obtained by the severance of the caudal peduncle. Samples were frozen and stored at -20°C until analysis. Liver samples were taken for the determination of liver glycogen and protein levels.

Thyroid, liver, kidney, head kidney, gill, spleen, cartilage and blood smears were subjected to routine histological analysis.

Field studies

The Hemlo gold mining region of northern Ontario was selected as a site for the environmental health assessment study of the impacts of SCN^--bearing effluents on aquatic systems. Effluent effects on fish populations will be assessed by monitoring growth (age versus size relationships), reproductive capacity (size at sexual maturity, fecundity, egg size, hatchability), histopathology of major tissues and biochemical parameters shown in the laboratory to be affected by SCN^- exposure.

Results

Acute baseline bioassay

The 96-h LC50 for unstressed rainbow trout exposed to KSCN was 250 mg/L. When mortalities due to the stress of a 15 s pursuit with a dip net after the 96-h exposure were included in the calculation, the 96-h LC50 was 180 mg/L. All fish that died after application of the stressor exhibited flaring of operculae, extreme muscle contraction resulting in a dramatic curvature and arching of the body, spasms, loss of equilibrium, loss of buoyancy control and changes in pigmentation, all signs characteristic of Sudden Death Syndrome (SDS).

Chronic exposure studies

Mortalities and behavioural anomalies

All fish exposed to 160 mg SCN^-/L died by the end of the 12 weeks. Many of the deaths coincided with the expression of SDS after bimonthly weighing procedures. Approximately 40% of the fish exposed to 120 mg SCN^-/L had died by the end of the 16 week growth trial, with varying proportions of the fish expressing SDS after weighing. Mortalities at the two lower concentrations (40 and 80 mg/L) were minimal, except for one replicate (80 mg/L) which was situated near a corner of the experimental system, and as such, was often disturbed by laboratory traffic. Increased mortalities in this replicate may suggest an effect of an outside stressor on the expression of SCN^- toxicity. Fish exposed to lower concentrations of SCN^- did not express SDS at any time.

The major behavioural anomalies observed were irritability and skittishness. Fish exposed to 120 mg SCN^-/L exhibited a behaviour in which fish swam rapidly in small circles for 10-20 s. This behaviour ususally coincided with the fish being offered pelleted food and attempting to feed.

Gross physical observations

All exposure levels of SCN^- resulted in varying degrees of deformities in the cranial region of the trout. This condition was characterized by the small size of the head of the trout in relation to the body. The head was laterally and dorso-ventrally compressed. Operculae were often shortened or crumpled, exposing gill filaments. The severity of these signs appeared to increase with SCN$^-$ concentrations.

Pigmentation changes were also evident as the darkening of individual fish within tanks receiving thiocyanate. This darkening appeared to be transitory, as all fish in tanks where dark fish were observed would be lighter in colour upon subsequent observation. Liver somatic and splenosomatic indices decreased in exposed fish and the livers of fish exposed to 120 mg SCN$^-$/L appeared pale and were friable.

Blood parameters

Fish exposed to SCN$^-$ developed an anemia characterized by decreases in hemoglobin levels and hematocrit. Further histological characterization of cell types in blood smears is currently underway. The decreased hemoglobin and hematocrit in conjunction with the decreased splenosomatic index are suggestive of a hypoplastic anemia. The magnitude of the decreases in hemoglobin and hematocrit increased at higher SCN$^-$ concentrations. Plasma total cyanide-reactive substances increased with waterborne SCN$^-$ concentrations. Plasma samples are currently being evaluated for tri-iodothyronine (T_3) and thyroxine (T_4) levels.

Tissue parameters

The evaluation of tissues for various parameters is currently underway. Liver will be analyzed for glycogen and protein. Routine observations during sampling suggest that there may be more visceral fat present in fish exposed to thiocyanate.

Carcass samples have been frozen for routine proximate analysis of fat, protein, ash and moisture.

Histology

Liver, head kidney, kidney, spleen, thyroid and cartilage have been sampled and fixed for histological analysis.

Discussion

Based upon preliminary analysis of data, long-term, sublethal exposure of rainbow trout to SCN^- results in a syndrome characterized by decreased growth and feed intake, cranial deformities and a hypoplastic anemia. Plasma total cyanide-reactive substances are also elevated with increasing waterborne SCN^- concentrations. Sublethal physiological and morphological effects were noted at all concentrations of SCN^- tested, although there appeared to an increase in the incidence and magnitude of responses with SCN^- concentration.

The sublethal mode of action of SCN^- is not well understood in fish. The sublethal effects of SCN^- in mammals are exerted by its antithyroidal activity, inhibiting the active uptake of iodine from the blood by the thyroid and acting to uncouple T_3 and T_4 from their carrier proteins in the blood (Green, 1971). Thiocyanate also actively competes for membrane transport with other halides such as chloride (Epstein et al., 1975; Katz et al., 1982), and hence could be involved in ionoregulatory disruptions. How these potential modes of action fit into the sublethal toxicity syndrome seen in this study, remains to be determined, pending final analysis of data.

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B5

POTENTIAL ROLE OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE DEVELOPMENT OF LIVER TUMORS IN FISH FROM POLLUTED SITES OF LAKE ONTARIO.

G.M. Kirby, I.R. Smith*, C. Thorn, H.W. Ferguson and M.A. Hayes
Fish Pathology Laboratory, University of Guelph, Guelph, Ontario N1G 2W1
*Ontario Ministry of Environment, Rexdale, Ontario, Canada, M9W 5L1.

ABSTRACT

Various liver tumors occur with increased frequency in several species of bottom-dwelling fish inhabiting locations with industrially polluted sediments. In white suckers *(Catostomus commersoni)* from the Hamilton Harbour region of Lake Ontario, preneoplastic and neoplastic liver and bile duct neoplasms are well recognized but are much less prevalent in white suckers from less-polluted control sites in Lakes Huron and Simcoe. Polyclonal antiserum prepared against purified hepatic glutathione S-transferases (GSTs) from white suckers was used for immunocytochemical demonstration of GST expression in the various liver lesions in white suckers. GSTs are considered to be important in detoxification of polycyclic hydrocarbons (PAHs) and diminish the mutagenicity of benzo(a)pyrene (BaP). All liver tumors were GST-deficient in comparison with surrounding non-neoplastic liver. Also, these fish had few early preneoplastic foci compared with advanced liver tumors. White Suckers from the western Lake Ontario region had faster rates of biliary excretion of BaP than fish from clean sites in Lake Huron. These findings suggest that environmental mutagenic chemicals such as PAHs, which can be normally detoxified by GSTs in these fish, may play a role in the later stages (malignant progression) of cancer development in cells that have lost GST-dependent resistance mechanisms. This hypothesis implies that long-term exposure to PAHs may necessary to cause neoplasms in tissues that are initially resistant to them.

INTRODUCTION

The Hamilton Harbour region of Lake Ontario, like many other industrial sites in the Great Lakes, has a wide range of organic chemical pollutants accumulated in the sediments (Harlow and Hodson, 1988). In recent years, increased prevalences of various skin and liver neoplasms have been demonstrated in bottom-dwelling fish from the Hamilton Harbour region, especially in White Suckers (*Catostomus commersoni*) (Sonstergard and Leatherland, 1984; Smith and Ferguson, 1986; Metcalfe *et al*, 1987; Hayes *et al.*, 1987; V. Cairns, personal communication). The geographic association of these tumours with increased industrial pollution suggests that the affected fish may be exposed to carcinogenic pollutants, but the implied cause-effect relationship has not been clearly demonstrated. Because there are numerous possibly carcinogenic agents in the sediments to which the affected fish are exposed (Harlow and Hodson, 1988), it is unlikely that any one class of chemicals can be definitively and exclusively implicated by epidemiological studies. However, the abundance of various polycyclic aromatic hydrocarbons (PAH) in the polluted environment (Harlow and Hodson, 1988), many of which are known skin and hepatic carcinogens for mammals and fish (Slaga *et al*, 1980; Hendricks *et al*. 1985) supports a reasonable suspicion that PAHs may play a role in the development of the neoplasms observed in these fish. This suspicion is reinforced by evidence that similar neoplasms in other benthic fish in Puget Sound (Meyers *et al*., 1987; Varanasi *et al*., 1987) Lake Erie (Baumann and Harshbarger, 1985) and Lake Ontario (Metcalfe *et al*, 1987; Dunn *et al*., 1988) are associated with increased exposure to PAHs.

Our recent studies have addressed the question that PAHs might be responsible for the liver tumours we have observed in White Suckers from the Hamilton region. We have been comparing the kinetics and metabolism of benzo(a)pyrene (BaP) as a typical carcinogenic PAH in fish from the Hamilton region and from less polluted reference sites in Lake Simcoe and Lake Huron. These studies suggest that fish from the polluted site have an induced ability to activate BaP in the liver and to excrete it in the bile as glutathione (GSH) conjugated metabolites. Furthermore, preliminary evidence indicates that the liver in in White Suckers may be naturally resistant to PAHs because of the normal hepatic glutathione S-transferases (GSTs) which are important detoxification enzymes for PAHs in these fish. Developing liver neoplasms lose their normal GST enzymes and thereby likely become more sensitive to the PAHs that would be adequately detoxified and excreted by GSTs in normal liver cells . Collectively, our observations support a hypothetical explanation for carcinogen-induced genetic alterations responsible for malignant transformation and cancer progression in cells that may have been originally quite resistant to PAHs and other carcinogens. An important aspect of this emerging concept is that repeated exposures to high doses of genotoxic carcinogens may cause malignancies in resistant tissues, individuals or species.

METHODS

1. Distribution and Excretion of Benzo(a)pyrene (BaP)

Male White Suckers were captured from Sixteen Mile Creek near Oakville, Lake Ontario (polluted site) and from Keefer's Creek, Lake Huron (reference site) during their spring spawning migrations and were maintained in laboratory holding tanks in clean well water. Fish were selected and given BaP (2 mg/kg) by gavage in a vehicle composed of distilled deionized water, DMSO (10%) and sodium deoxycholate (1%). The BaP preparation contained 50 µCi/mg of ^{3}H-BaP (New England Nuclear, Boston MA) as a radioactive tracer. Treated fish were killed after 3, 6, 12 and 24 hours and subjected to postmortem examination, during which samples of bile (gall bladder), liver, muscle, blood, kidney, intestine, intestinal contents and gill were collected for liquid scintillation counting (LSC) of the ^{3}H-tracer.

In a second experiment, White Suckers from Oakville were held for 6 weeks under clean laboratory conditions before BaP administration to determine if the rates of BaP metabolism were altered when the fish were no longer exposed to their polluted natural environment.

Samples of blood and bile (40 µl) were solubilized in 1 ml of Protosol (NEN) and counted in 10 ml of Aquasol (NEN). Samples of tissue (100 mg) were digested and extracted overnight in 2 ml 0.5N NaOH and 3 ml n-hexane on a motorized rotater (Varanasi *et al.*, 1978). An aliquot (1.5 ml) of the n-hexane fraction containing non polar BaP metabolities and the NaOH fraction (1 ml) containing polar metabolites were then counted in 10 ml of Aquasol. Concentrations of BaP and derivatives were calculated as nmol/g tissue from liquid scintillation counts of vials after overnight dark-acclimation and correction for counting efficiency.

2. Metabolism of BaP

Samples of bile obtained from fish given ^{3}H-BaP were subjected to enzyme hydrolysis and HPLC analysis to determine the proportions of B(a)P excreted as polar metabolites and conjugates. Bile in 1 ml of distilled water was extracted initially with ethyl acetate to remove non polar BaP (parent compound). The residual aqueous phase was divided into 3 equal parts brought to 0.9 mls using sodium acetate buffer, incubated with arylsulfatase (Sigma Chemical Col., St. Louis, MO, 35 units/ml incubate containing 20 mM D-saccharic acid 1-4 lactone to inhibit glucuronidase activity) or β-glucuronidase (Glucurase,Sigma) or sodium acetate buffer (0.2 mM, pH 5.0, 37°C, for 24 hrs). Each sample was further extracted with ethyl acetate (2 x 2 ml) to remove less polar hydrolyzed BaP intermediates. All extracts and fractions were subjected to LSC to determine percentages of hydrolysible and nonhydrolysible polar metabolites. Samples of bile (50 µl) from fish given ^{3}H-BaP 24 hours previously were analyzed by reverse phase HPLC using a stepwise water/methanol gradient on a C18 column (Biorad Hipore RP-318; 250 x 4.6 mm) on a Biorad 402 HPLC system. The mobile phase conditions were 0-30% methanol in 2 minutes, 30% methanol for 15 minutes,30-70% in 3 min and 70% methanol for 10 minutes at a flow rate of 1.0 ml/minute. The eluant was monitored by absorbance (430 nm) and also by fluorescence (excitation 380 nm; emission 430 nm) by a Shimadzu RF 5000 spectrofluorometer and collected as 0.25 ml fractions in a Gilson Model 203 microfraction collector. The distribution of ^{3}H radioactivity tracer in fractions was determined by LSC.

3. Determination of GST Activity and Expression in Fish Tissues

Samples of liver, kidney, muscle, intestine and gill were also collected for histopathologic examination (fixed in 10% formalin). Samples of liver were also collected on ice, and homogenized in 3 volumes of 0.25 M sucrose buffer (containing 50 mM HEPES pH 7.5) for determination of hepatic glutathione S-transferase (GST) activity. Liver homogenates were centrifuged at 100,000 g for 1 hour to obtain cytosol (supernatant) from which GST activity was determined by CDNB conjugation rates (pH 7.0, 30°C) by the method of Habig *et al.* (1974). Protein concentrations in cytosol were determined by the Lowry method.

GST isoenzymes were purified from liver cytosol from normal White Suckers by affinity binding to S-hexylglutathione-agarose (Sigma) and stepwise elution with 50 mM and 200 mM NaCl (to remove non specifically bound proteins) and then 5 mM S-hexylglutatione (to elute glutathione-binding cytoslic proteins). The latter fraction, containing approximately 80% of hepatic GST, was analyzed by SDS-PAGE under reducing conditions and was found to consist of 4 major protein subunits in the 26 kD molecular weight range corresponding to reference samples of pure GSTs from rat liver. The purified GSTs were used to immunize rabbits to produce polyclonal antiserum specific for all 4 GST subunits in hepatic cytosol (by western blot analysis). This specific antiserum was used in routine peroxidase-antiperoxidase (PAP) immunocytochemical staining for GST protein expression in formalin-fixed sections of livers from various Oakville and Hamilton Harbour fish with previously diagnosed hepatocellular or bile duct neoplasms.

RESULTS

1. Distribution and Excretion of BaP

Fish from Oakville excreted BaP twice as fast as reference fish when they were exposed to ^{3}H-BaP (2mg/kg) within 7 days of capture from the wild (Figure 1). The vast majority of polar BaP metabolites were found in the bile. BaP-derived radioactivity in muscle, blood,gill, and kidney were negligible in both populations (less than 1.0 % of total) (Figures 2 & 3). Large amounts of ^{3}H activity were present in liver and intestine. However, further analysis of these latter samples by NaOH/n-hexane extraction (see below) revealed that the intestine contained mainly unabsorbed parent compound whereas the liver contained mainly polar metabolites of similar types to those found in the bile.

The fish from Oakville exposed to BaP after 6 weeks in captivity excreted BaP at a lower rate similar to that observed in fish from the reference site at the time of capture (Table 2).

2. Metabolism of B(a)P

The majority of radioactivity in bile occurred as more polar metabolites that could not be extracted from the aqueous phase by ethyl acetate. The aqueous phase of bile from Oakville and reference fish contained some metabolites that were hydrolysed by aryl sulfatase or β-glucuronidase (Table 3). These experiments indicated that Oakville fish had an increased proportion of sulfated conjugates (16.2 fold), but a similar proportion of glucuronides as reference fish (Table 3). Non hydrolysible polar metabolites, presumably containing glutathione and other conjugates, comprised a substantial proportion of the ^{3}H-BaP in bile from each group.

The distribution of fluorescent and ^{3}H-labelled metabolites of BaP in HPLC analyses of whole unextracted bile indicated that fish from polluted and reference sites had a similar complex profile of moderately polar and highly polar BaP metabolites (Figures 2,3). No parent (non polar) BaP was detectable in these bile samples. While there were minor, as yet uncharacterized, differences in some of the metabolites, the observations were consistent with excretion studies (Table 1) which indicated an overall increased rate of BaP metabolism and elimination of BaP in bile.

3. Determination of GST Activity and Expression in Fish Tissue

Activities of GST in hepatic cytosol from White Suckers exposed to BaP in these experiments were substantially lower than GST activities in White Suckers that had not been given BaP (Table 4). These observations are consistent with an interpretation that BaP metabolites compete with or inactivate GST in liver. Fish from Oakville had approximately one half the GST activity when compared with fish from the reference site.

Immunocytochemical stains of tissues from White Suckers revealed that GST proteins are present in substantial amounts in hepatocytes, bile duct epithelium, gill epithelium, intestinal epithelium, renal tubules and erythrocytes. All hepatocellular and bile duct adenomas or carcinomas examined in fish from Hamilton Harbour or Oakville were markedly deficient in GST proteins in comparison with surrounding non-neoplastic hepatocytes or major bile ducts. Preneoplastic hepatocellular lesions (foci of altered hepatocytes) which were rarely observed in livers of fish with neoplasms, on the whole had similar amounts of GST as did normal hepatocytes, but occasionally, GST deficient foci of hepatocytes were observed. No preneoplastic or neoplastic lesions with induced GST were observed.

DISCUSSION

These studies indicate that White Suckers from the industrially polluted western region of Lake Ontario have evidence of an induced ability to metabolize and rapidly eliminate BaP in the bile. This induction is transient and subsides when fish are held for some time in an uncontaminated environment. This evidence supports the view that these fish may be exposed to xenobiotics that induce various hepatic cytochromes P-450 and detoxification enzymes involved in elimination of xenobiotics. A reasonable interpretation of these findings is that fish are influenced by the polluted environment in western Lake Ontario, and that this influence helps them to excrete greater amounts of PAHs than can fish from less polluted regions.

The observations that White Suckers have numerous biliary metabolites of BaP are in accordance with observations that English sole from Puget Sound also excrete BaP by multiple pathways (Varanasi *et al.*, 1987). Our evidence suggests that conjugation with GSH by hepatic GST activity is a major detoxification mechanism for BaP in White Suckers. Because these fish are capable of rapidly excreting relatively large experimental dosages of BaP, especially when they have lived near or in the Hamilton Harbourthat is contaminated with many PAHs (Harlow and Hodson, 1988), it is reasonable to consider that White Suckers are relatively resistant to BaP by virtue of their natural GST and other detoxification pathways. Such an interpretation is consistent with our observations (Hayes *et al*, 1987) and those of others (V.Cairns, personal communication))that hepatic neoplasms are observed in fewer than 10% of White Suckers that have likely been exposed to the Hamilton environment for many years. Moreover, preneoplastic foci of altered hepatocytes, of the type considered to be initiated by PAHs and other genotoxic carcinogens in rodents (Farber and Sarma, 1986) and fish (Hendricks *et al.* 1985; Meyers *et al.* 1987), were rarely observed in these fish inspite of their presumably chronic exposure to PAHs and other potentially genotoxic carcinogens. Accordingly, it is reasonable to conclude that White Suckers exposed to the Hamilton environment are rather resistant to any initiating effects of the PAHs to which they are exposed.

Because there is an apparently high rate of malignant progression (conversion) of preneoplastic lesions to hepatocellular adenomas and carcinomas, these fish must develop an increased susceptibility to tumorigenesis at some stage after the initiation step. In laboratory rodents, few of the numerous foci of altered hepatocytes initiated by brief exposures to genotoxic carcinogens actually progress to malignancy (Farber and Sarma, 1986). By comparison, preneoplastic epidermal papillomas initiated by PAHs in mice exhibit a high rate of malignant conversion when they are subsequently exposed repeatedly to genotoxic carcinogens, including PAHs (Hennings *et al*, 1983). One of several reasonable explanations for the consistent GST-deficient phenotype in neoplasms that have progressed in White Suckers is that multiple subsequent "hits" by PAHs or other carcinogens may have been involved in the later stages of carcinogenesis. Because all advanced hepatic neoplasms had markedly reduced GST resistance, it is plausible that loss of GST expression in rare initiated cells would render them more prone to further DNA damage by agents such as BaP for which normal hepatocyte GSTs are protective.

This hypothetical concept that a reduction in normal cellular resistance to carcinogens could favour tumour progression is important from an epidemiological viewpoint. Our findings suggest that environmental mutagenic chemicals such as PAHs, which can be normally detoxified by GSTs in these fish, are perhaps more likely play a role in the later stages (malignant progression) of cancer development in cells that have lost GST-dependent resistance mechanisms. This hypothesis implies that long-term exposure to PAHs may necessary to cause neoplasms in tissues that are initially resistant to them. This view also implies that some species or individuals with genetic deficiencies in specific protective mechanisms would be more susceptible to carcinogens that otherwise would be detoxified. Further investigation of this hypothesis and its implications for human suscepibility to the carcinogenic effects of environmental PAHs are currently underway. The availability of fish exposed continuously to PAHs under natural conditions provides a means of understanding the circumstances under which PAHs may be carcinogenic to humans. This knowledge is essential to a sound assessment of cancer risk in humans exposed to environmental PAHs in contaminated water, air or diet.

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Table 1 : ^{3}H BaP EXCRETION IN BILE AT 24 HOURS POST ADMINISTRATION

Reference	Polluted	Polluted (6 weeks)*
140.4	250.8	120.4

Values are expressed as nmoles of BaP equivalents/g bile
*White suckers held in clean water tanks for 6 weeks after capture
from polluted site.

Table 2: CONCENTRATION OF BaP METABOLITES IN BILE OF WHITE SUCKERS AS DETERMINED BY ENZYME HYDROLYSIS

Metabolic Derivative	Reference sites	Polluted sites	Polluted/Reference
Ethyl acetate extractable metabolites	20.2	20.7	1.2
Sulfates	0.6	9.7	16.2
Glucuronides	14.7	23.4	1.6
Other	22.6	45.7	2.0

Values expressed as nmoles of BaP/g bile

Table 3: GLUTATHIONE S-TRANSFERASE ACTIVITY IN LIVER OF BaP-TREATED AND NON TREATED WHITE SUCKERS

^{3}H-BaP-treated		Untreated
Polluted site	Reference site	Polluted site
0.59	1.08	2.76

Values expressed as IU/mg protein.

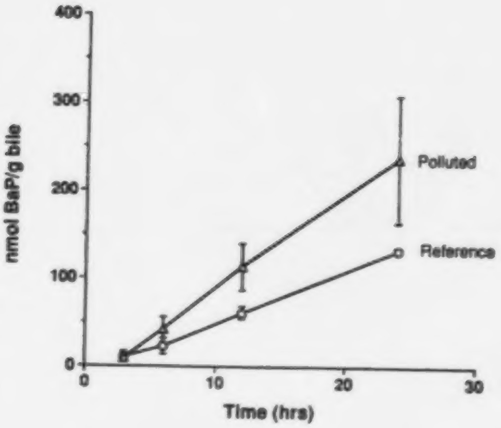


Figure 1:

Concentration of B(a)P in the bile of White suckers from polluted and reference areas at various times after oral dosing with B(a)P (2mg/kg).

Values determined by total radioactivity in NaOH extracts of bile (100 µl) expressed as nmol/g of B(a)P equivalents per gram of bile (mean ± SE)

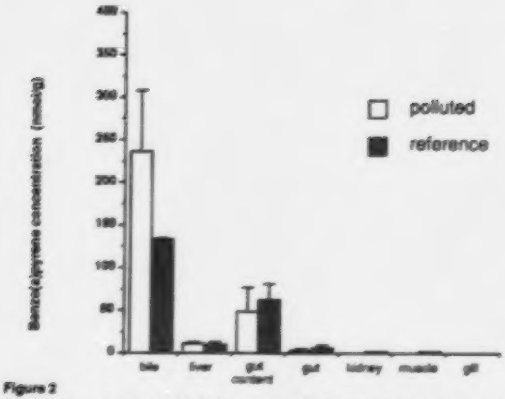


Figure 2

Concentrations of water-soluble BaP metabolites (NaOH extractable) in tissues and fluids of polluted and reference White suckers 24 hours after oral exposure to tritiated BaP.

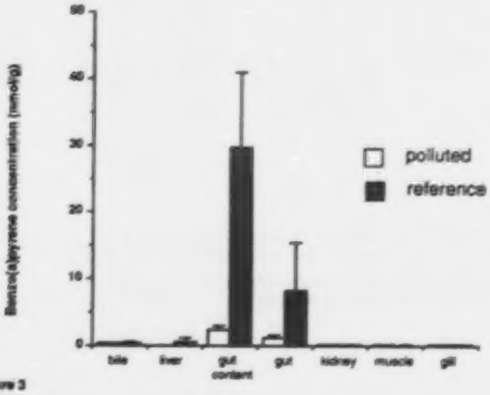


Figure 3

Concentrations of organic-soluble BaP metabolites and parent compound (n-Hexane extractable) in tissues and fluids of polluted and reference White suckers 24 hours after oral exposure to tritiated BaP.

Figure 4 Radioactivity of Chromatographic Fractions of Bile from White Suckers from Polluted Sites

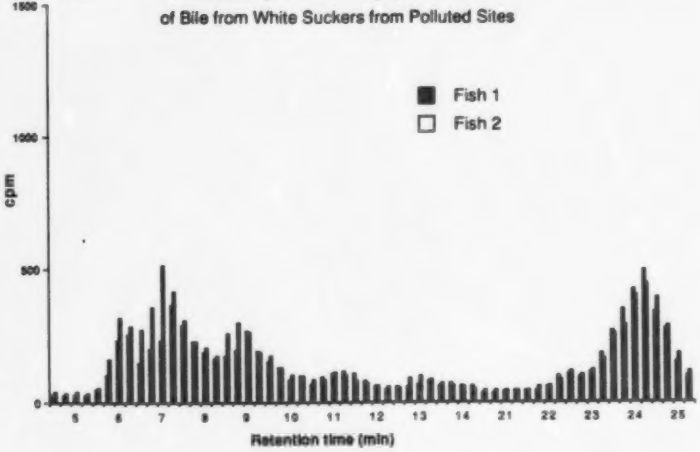
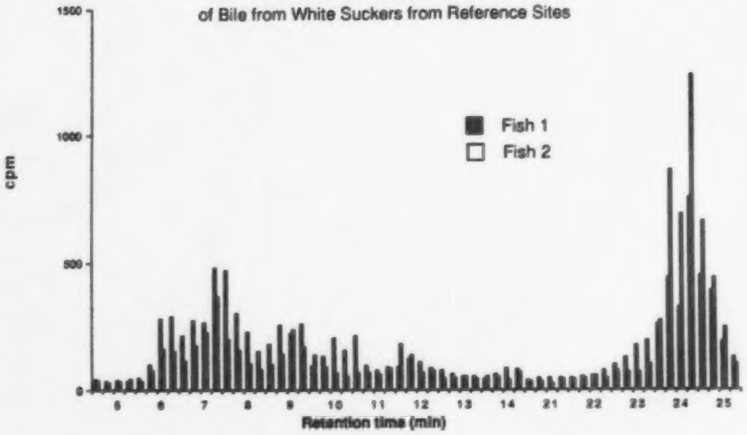


Figure 5 Radioactivity of Chromatographic Fractions of Bile from White Suckers from Reference Sites



B6

Preliminary Data on Plant Bioassays for the Detection of Environmental Mutagens in an Aquatic Environment

William F. Grant

Department of Biology, York University and
Department of Plant Science, P.O. Box 4000,
Macdonald College of McGill University,
Ste. Anne de Bellevue, Quebec H9X 1C0

ABSTRACT

Various higher plant assays have been developed for screening and monitoring airborne and aqueous mutagens (de Serres 1978; Grant 1978, Grant *et al.* 1981; Nilan 1978). These assays are inexpensive, easy to handle and applicable to indoor as well as outdoor detection of environmental mutagens. Quantitative plant assays for the genotoxic detection of aqueous pollutants are relevant and useful in establishing water quality standards. This project was initiated in order to have a different relatively inexpensive mutagen assay system (using higher plants) which would provide a measurement of mutagenic toxicity in assay systems not being carried out by the Ontario Ministry of the Environment. We are developing such an assay for genotoxic aqueous pollutants using two higher plants, namely, *Tradescantia*, and *Vicia faba*. Each plant assay has different features for detecting gene mutations and/or chromosome aberrations. *Tradescantia* has two assay systems: (1) the Stamen Hair Assay for the detection of gene mutations, and (2) the Micronucleus Test for the detection of chromosomal aberrations. The *Vicia faba* assay system detects chromosomal aberrations in root tips. These assays are being tested to valididate the assays under field conditions and to complement information provided by other mutagen assay systems being carried out by the Ontario Ministry of the Environment. This paper will give the preliminary results of a field trial carried out on effluent from a paper mill on Lake Superior, a test of the assays at Go Home Bay, Georgian Bay, and in a pond at York University. Using these assays, we are also collecting data on the mutagenicity of a number of dyes used in the paper industry.

Introduction

Higher plants provide valuable assay systems for screening and monitoring environmental chemicals, both gaseous and liquid (Grant *et al*. 1981). Although higher plant assays for the detection of mutagens have been in existence for many years, they have only recently received the recognition which these sensitive and reliable systems warrant (de Serres 1978; Grant 1978; Nilan 1978). As Stich and San (1980) stated "The recent successful introduction of the use of *Tradescantia* staminal hairs to detect airborne mutagens and carcinogens, may be the beginning of the recognition of various plant assays which are inexpensive, easy to handle and applicable to indoor as well as outdoor detection of environmental mutagens". Studies have shown that, for a specific chemical agent, comparable results in terms of genetic abnormalities are obtained in plant and animal systems. For example, in a survey of studies on the effects of pesticides, it has been shown that an excellent correlation exists between the frequency of both chromosomal abnormalities and C-mitoses in plant and animal systems (Grant 1978). A similar conclusion was drawn in studies on the effect of eight chemicals in several systems, including *in vitro* and *in vivo* mammalian systems, bacteria, *Drosophila* and plant systems. The plant systems showed excellent correlations with the mammalian systems (Clive and Spector 1978). These higher plant assays are being tested under field conditions and were initiated to complement information provided by other mutagen assay systems being carried out by the Ontario Ministry of the Environment. This paper will give the preliminary results of the stamen hair mutation assays for field trials carried out on effluent from a paper mill on Lake Superior, a test of the assays at Go Home Bay, Georgian Bay, and in a pond at York University. Data on the mutagenicity of a number of dyes used in the paper industry will be presented elsewhere. The goal of the study has been to test these assays under practical field conditions.

Materials and Methods

Study Sites

(a) A field trip was carried out to Go Home Bay, Lake Superior, July 4 and 5, 1988. Floats as described below were placed out at five locations and plants returned to York University for analyses.

(b) A field trip was carried out between July 19 and July 26, 1988, to the north shore of Lake Superior, for testing the water of Blackbird Creek, Moberly Bay, and Jackfish Bay. These sites are contiguous waterways and receive the effluent discharged from a paper mill. The study was designed to test for mutations and chromosome aberrations

using *Tradescantia* and *Vicia faba* by growing the plants in these waterways. Waterways near the test sites were used as a control sites.
(c) The Tradescantia stamen assay was also carried out in a pond at York University.

Floats

A float for holding both *Tradescantia* plant cuttings and *Vicia* germinating seedlings has been designed for aqueous testing in different types of aquatic habitats (still, slow moving and fast running water). The float is 350 cm in diameter and consists of a 265 cm circular plexiglass disk with a central circular enclosed box 10 cm in diameter X 4 cm deep which is air tight, with a 4 X 3.5 cm ring of styrofoam encircling the disk for buoyancy. A circular plexiglass rod, 1.25 cm diameter and 45 cm in length, may be attached by means of a screw to a holder on the bottom center of the box on the disk for stability. The plexiglass disk is drilled with holes for holding 30 *Tradescantia* cuttings. A 50 X 60 cm plastic pot is attached to the plexiglass disk by means of plant ties. This plastic pot is for holding the germinated seeds of *Vicia faba* during testing.

Higher Plant Systems

(1) *Tradescantia* clone 4430, heterozygous for flower color, blue dominant to pink recessive, developed at the Brookhaven National Laboratory originally for the study of the effects of irradiation (Underbrink *et al*. 1973)
Assays: (a) The staminal hair assay system will detect air borne, soil, and aqueous mutants (Ichikawa, S. 1978; Schairer et al. 1978)
(b) The micronucleus assay will detect chromosome aberrations (Ma 1981).

(2) *Vicia faba* (broad bean) Assay:

Root tips for the detection of mitotic chromosome aberrations (Ma 1982).

Protocols

Protocols have been fully developed for both the Tradescantia and Vicia assays. A brief outline of these assays is given below and in greater detail in Appendix Tables I, II, and III.
Results on the *Tradescantia* stamen hair assay will be reported here.

Tradescantia

The Tradescantia *Stamen Hair Assay* is highly sensitive to chemical mutagens. In the Stamen Hair Assay, inflorescences of the appropriate age are placed in a drop of paraffin oil and observed under a dissecting microscope. Pink mutant cells are scored. Details of the protocol are given in Appendix Table I.

For the Tradescantia *Micronucleus Assay*, pollen mother cells which are highly synchronized are examined in the quartet (tetrad) stage (about 24 hours prior to anthesis) and the number of micronuclei are scored. The micronuclei reflect chromosome aberrations which occurred at earlier stages in meiosis. The frequency of micronuclei per 100 cells can be used as an index of mutation. Details of the Protocol are given in Appendix Table II.

Vicia faba

Vicia faba seed are germinated in petri dishes in an incubator at 22 C. Seeds are treated and root tips of young seedlings are examined for chromosome aberrations. The protocol for the Vicia *faba* Chromosome Aberration Assay is given in Appendix Table III.

The floats holding the *Tradescantia* cuttings and the *Vicia faba* root tips were left in the test sites for 24 hours after which the cuttings were removed and placed in beakers containing Hoagland's nutrient solution and the root tips were fixed in 3:1 (3 parts 95% ethanol: 1 part glacial acetic acid). The solution containing the cuttings was changed daily until the cuttings were placed in a growth chamber and data subsequently taken as per protocol.

Results to date

Tradescantia Stamen Hair Mutation Assay

(a) *Go Home Bay*, Georgian Bay: The five locations were as follows:

1. Below the falls,
2. Inlet,
3. Open water behind island
4. Open cove
5. Cove off channel

Table 1 gives the results of the Stamen Hair Assays. The results are similar with a mean of 3.01 + 0.28. The sample from the Cove is higher than all the others; perhaps this may be explained by being closer to the shore. All of the results are higher than our Laboratory water control which averages around 1.01.

(b) Lake Superior

Table 3 gives preliminary data on mutations from the field trial in Moberly Bay and Jackfish Bay (Lake Superior). The sites (1 to 6) are in increasing distance from the mouth of Blackbird Creek where the mill waste effluent empties into Moberly Bay. The water temperatures during the treatment period ranged from 15 to 18°C and the air temperatures from 17 to 21°C. The pH was determined of collected samples and ranged from 5.97 to 6.34.

Results of water samples taken from the sites will be presented at the Technology Transfer Conference.

(c) Stong Pond, York University

Two locations were selected designated the upper and lower pond locations. The plants were divided into two experiments, in light and dark in which the "dark plants" were grown in the dark for 48 hours prior to use. This was to determine the effect of travelling with plants in a van for 48 hours prior to the plants being placed in a growth chamber for mutation analyses. The results from the "dark" treatment had a greater range in contrast to the "light" treatment, but the results from both treatments were higher in the lower pond.

Conclusion

The purpose of the the present experiments was to test the higher plant assays under actual field conditions. The data analyzed to date indicate that the *Tradescantia* stamen hair assay is reliable and can be used to complement mutagenic/toxicity assay systems being carried out by the Ontario Ministry of the Environment. Data on the micronuclei assay, *Vicia faba* chromosome assay and water samples are still being analyzed and will be presented at the Technology Transfer Conference.

Acknowledgements

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Table 1. Tradescantia Stamen Hair Mutation Assay -
Go Home Bay, Georgian Bay Test Sites

Day	Below Falls		Inlet		Open Water		Open Cove		Cove	
	No. flowers scored	No. pink events	Flowers	Pinks	Flwers	Pinks	Flowrs	Pinks	Flwers	Pinks
4	10	10	10	11	10	10	15	11	9	10
7	6	32	2	3	5	6	4	69	2	18
8	2	23	4	44	4	6	4	17	6	48
9	4	54	9	121	4	17	2	20	7	121
11	7	56	9	26	4	39	4	57	7	126
14	6	75	6	84	8	115	4	22	6	171
15	1	19	3	57	3	53	1	9	1	28
16	3	86	5	98	4	49	2	30	3	97
17	4	36	2	44	5	61				
18	4	53	3	34	7	75	3	19		45
21	4	83	8	143	5	59	1	7	1	10
22	1	29	1	6	2	21	3	31		
23	3	25	1	9	2	40			1	24
24					3	84			1	6
Total	55	581	63	680	66	685	43	286	49	704
a)		1.76		1.80		1.73		1.11		2.39
b)		50.00		46.00		51.00		39.00		54.00
c)		3.5		3.9		3.4		2.85		4.43

a) = Mean pinks/stamen; b) = Mean hairs/stamen; c) = Pink mutations/100 hairs

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Lab standard = 1.01 ± 0.62 mutations/100 hairs.

Table 2. Tradescantia Stamen Hair Mutation Assay -
Moberley Bay and Jackfish Bay (Lake Superior)

Mean pinks/stamen						
			Sites			
Control	1	2	3	4	5	6
2.39	3.54	4.38	3.60	3.04	3.02	3.00

Table 3. Tradescantia Stamen Hair Mutation Assay -
Stong Pond, York University

Mean pinks/stamen			
	Sites		
Upper pond/light[a]	Lower pond/light	Upper pond/dark[b]	Lower pond/dark
1.24	1.28	0.89	1.31

[a] Tradescantia plants grown in the light in the greenhouse.

[b] Tradescantia plants grown in the dark 48 hours prior to use.

APPENDIX

TABLE II. Protocol for the *Tradescantia* Micronucleus (MCN) Bioassy

The meiotic pollen mother cells (PMC) of *Tradescantia* are highly synchronized and are very sensitive to physical and chemical mutagens (Ma, 1981). A high frequency of acentric fragements can be induced with very low level of mutagens. These induced chromosome aberration become micronuclei (MCN) in the synchronized tetrads and they can be easily identified and scored.

The MCN Bioassy involves:

a) Making cuttings of young inflorescences prior to meiosis. Grow cuttings in aerated Hoagland's nutrient solution.

b) Exposure of PMC in the young inflorescence of the plants by placing the cuttings in the test chemical and allowing the liquid to absorb through the stem to the inflorescence just prior to the onset of meiosis.

c) Fix flower buds 9 to 12 days after exposure (testing one or two buds prior to fixation to confirm that meiosis has proceeded to the tetrad stage) in acetic-alcohol (1:3).

d) Prepare microslides by removing a few PMc's at a time and squashing the cells in aceto-carmine stain. The stain penetrates fairly readily, but flaming over an alcholol lamp briefly will speed the staining process.

e) Cytological Examination: The tetrads are examined at a magnification of 400X (10 X ocular and a 40 X objective).

f) Statistics: An examination of 300 tetrads per slide and five slides per treatment have been found to give highly significant results.

A complete description of the technique with illustrations is given in the paper by Ma (1981). Earlier references are given in Ma's paper.

APPENDIX

TABLE I. Protocol for the *Tradescantia* Stamen Hair (ST) Assay

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The *Tradescantia* Stamen Hair Assay was developed by A. H. Sparrow of Brookhaven National Laboratory for the study of the effects of ionizing radiation. Early radiobiological data demonstrated that stamen hairs were sensitive to as little as 0.25 rad of x-rays.
Stich and San (1980) have stated "The recent introduction of the use of *Tradescantia* staminal hairs to detect airborne mutagens and carcinogens may be the beginning of the recognition of various plant assays which are inexpensive, easy to handle and applicable to indoor as well as outdoor detection of environmental mutagens."

For environmental chemicals, the *Tradescantia* clone 4430 has been developed at the Brookhaven National Laboratory. This clone is heterozygous for flower color and staminal hairs. Mutations are detected by the change in color from blue to pink.

The Staminal Hair Bioassy involves:

a) Making cuttings of young inflorescences prior to meiosis. Grow cuttings in aerated Hoagland's nutrient solution.

b) Expose the young inflorescences by placing the cuttings in the test chemical and allowing the liquid to absorb through the stem to the inflorescence. One flower will develop about 400 stamen hairs.

c) Collect flowers daily from 8 to 20 days after treatment (if chemical causes severe toxic effect flower development may be delayed, then examine as long as 25-30 days after treatment). Put flowers in a container with moistened sponge in refrigerator until ready to examine.

d) Prepare slides by placing a stamen in liquid parafin for microscopic examination; add cover slip. Examine immediately if possible, or place in refrigerator for same day examination.

e) Cytological Examination: Examined at a magnification of 400X (10 X ocular and a 40 X objective). Use white light source (no filters) for the detection of pink mutations.

f) Statistics: Examine and score as in the following example: Score 10 stamens per treatment per day from 10 different cuttings. Score daily as above. Mutations are recorded as pink events per 100 or 300 stamens.

A complete description of the technique with illustrations are given in the paper by Schairer et al. (1978) and Underbrink et al. (1973). References are given at the end of the report.

APPENDIX

TABLE III. Protocol for the *Vicia faba* Assay in Environmental Monitoring

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Vicia faba has long been used for cytological and radiobiological studies (Read 1959). This species possess six pairs of chromosomes which are designated according to centromere position as either M (median) or S (terminal). The single pair of M chromosomes is more than twice the length of the S chromosomes (mean 2.3:1) and possesses a large satellite on the short arm.

Procedure:

In general seeds of *V. faba* are fairly large and do not lend themselves to germinate in a petri dish. Various techniques have been developed for their germination and culture.

1. Germination: Seeds may be soaked for 6-12 hours in tap water, then allowed to germinate in moist Perlite or Vermiculite or between paper towels, or cotton, or in running thermally regulated tap water (Grant et al. 1981) for 4 days at 20 C. When the primary roots are between 3 and 5 cm-long, the seed coats should be removed and the shoot tip cut off. Also, the tips of the primary roots should be cut off to stimulate growth of secondary lateral roots.

2. Chemicals: Test liquids should be prepared fresh, or if appropriate, are stored in a refrigerator and brought to room temperature ca. 1 hour before use. Test liquids are changed every day (evaporated amounts may be replaced).
Since chemistry of compounds vary in mode of action (act at different stages of the cell cycle, S-phase or during mitosis), duration of the mitotic cycle plus period of DNA synthesis should be determined (e.g. 20, 18.8, 23 h mitotic cycle).

3. Treatments: For treatments, the seeds are placed on the top of test tubes, or on screens over beakers (Grant et al. 1981), containing the test solutions so that the roots are submerged. Treatment times from 2 - 24 hours are most common with a series of 2, 4, 8, 12, 24 hours used to determine toxicity and threshold levels.

4. Temperature: The experiments should be performed at a relatively constant temperature ca. 20 C for the duration of the experiment. The experiment should be carried out in the dark for the treatment period.

5. Fixation: 3 parts 95% ethanol: 1 part glacial acetic acid, prepared freshly for each fixation. Fix 30 min to 24 hours, then wash and transfer to 70% ethanol for storage in a refrigerator. (Fixation and staining will suffer after two months of storage).

6. Staining: The Feulgen procedure is specific for DNA. The roots are removed from the fixative and first transferred to distilled water. After 2-5 minutes in water, roots are hydrolized.

a) Hydrolize in 1 N HCl at 60 C for 5 min (4 - 12 min varying with conditions; after storage of tissue in alcohol, the hydrolysis time may need to be extended).

b) Stain in Feulgen reagent for 2 hours (1 -3 hours) in the dark.

7. Maceration: Treat with 5% pectinase for 1-3 hours (if left in pectinase too long, root tips will become soft and difficult to handle, but if not long enough the cells will not spread).

8. Slide Preparation: On a slide remove darkly stained meristematic region from the rest of the root and squash in a drop of 45% acetic acid. Mount with a coverslip and make a temporary seal with paraffin, clear nail polish, or rubber cement.

9. Permanent Slides: Temporary slides will deteriorate after a few days. To make permanent place slide on dry ice and when preparation is frozen, coverslip is popped off with a razor blade or scapel under one of its corners. Quickly immerse slide in 2 changes of absolute alcohol and mount with euparol or other mounting medium and mount with a clean coverslip.

Macroscopic Parameters:

1). Turgescence - hardness of root tips; if high toxicity, roots will die; preliminary tests necessary to choose for experiment.

2). Changes of color - root tips as well as whole plant may change color from treatment with certain salts.

e.g. blue-green from copper sulphate,

e.g. brownish due to toxic effects causing cell death.

3). Root Form: a) C-tumour (3-5 days)

b) bending of roots or root tips.

4) Root Length: Compare with controls.

Microscopic Parameters:

1). Mitotic Index: Number of dividing cells (all stages of mitosis) per 1000 observed cells.

2). Aberrations of various types -

Scoring:

Mitotic Index, 1000 cell counts; 5 analyses per series. If MI too low, discard and go to new slide with good MI for scoring.

For Cytological Effects: ca. 500 cells scored per 100 cells per root tip

Statistics: Appropriate statistics to be carried out.

A complete description of the technique is given in the papers by Grant et al. (1981), Ma (1982) and Kihlman and Andersson (1984).

B7

EFFECTS OF TEMPERATURE AND FIELD PROCEEDURES ON PCB BIOACCUMULATION IN ELLIPTIO COMPLANATA

Al Melkic and Yves Rollin

Integrated Explorations
189 McCurdy Rd., Box 1385
Guelph, Ontario, N1H 6N8

INTRODUCTON

The fresh water clam Elliptio complanata has been used by the Ontario Ministry of the Environment for the past 9 years as a standard in-situ, bioaccumulating agent to detect trace contaminants in water. The continued use of this popular technique has made it necessary to address a number of questions regarding the environmental factors which may limit its practical application.

To investigate the environmental factors affecting clam bioaccumulation, we tested procedural variables of live clam transportation, deployment, tissue processing, and temperature. These tests were conducted simultaneously, during a single 21 day in-situ exposure experiment in the Niagara River at Niagara on the Lake. The uptake of PCB'S in E. complanata was used to evaluate the effect of these environmental variables.

The transport experiments involved maintaining clams at ambient and ice temperatures in both water and moist air. Deployment experiments included suspended, flat and compact cages, as well as support rings and sand boxes. Tissue processing tests included holding clams at ambient and ice temperatures for 8 hours and 24 hours before shucking and freezing.

Temperature experiments were conducted simultaneously with the in-situ tests. Water from the in-situ test area was continuously pumped to an adjacent building where its temperature was adjusted to provide a range of 5 to 25 degrees celcius at 5 degree intervals. This provided clams in the temperature experiment with a continuous supply of water from the same source as that of the clams being tested in the river.

METHODOLOGY

Field Methods

COLLECTION:
E. complanata specimens, measuring 6.5 to 7.2 cm, were collected from Balsam Lake, Rosedale Ontario and transported to the experimental site at Niagara-on-the-Lake within 5 hours, on October 6th, 1986. Details regarding the collection site, methods of transport, and the test procedures were documented in an earlier proceedings which reported preliminary results (Creese *et al.*, 1986).

TRANSPORT EXPERIMENT:
Clams were transported in contaminant free, food grade plastic bags in lake water maintained at the source temperature of 12 °C and also at ambient temperatures which ranged between 15 - 20 °C. Also kept at these tempeature regiemes were sets of clams that were kept moist but were not immersed in water. All clams except those acclimated for the temperature experiment were held for 48 hours at 12 °C. Thermally acclimated clams were stepped up or down in 5 °C increments every 12 hours until the desired temperature was attained.

TEMPERATURE EXPERIMENT:
An environmentally controlled system was established indoors in which 5 aquariums were maintained at 5, 10, 15, 20 & 25 °C receiving a constant flow of fresh, thermally adjusted water from the Niagara River. This was accomplished by chilling water in a reservoir to 4 °C and warming water in another to 26 °C then connecting them to a manifold system which combined flows from the resevoirs in various proportions to obtain the desired temperature increments. A flow of 4 l/min. was maintained through the 12 l. aquaria which were insulated with 2 inch thick styrofoam. In each aquarium 6 thermally acclimated and an equal number of non acclimated clams were supported upright in plastic rings measuring 2 inches in diameter and 2.5 inches high. The ring rested upon a wire mesh work to avoid sediment from being trapped within the rings. Temperature was monitored every 2 to 3 days for a 21 day period.

DEPLOYMENT EXPERIMENT:
Clams were deloyed on October 8th, 1986, on top of an underwater platform at approximately 3 meters depth. Five experimental deployment methods were tested; boxes containing sand from Balsam Lake, floating cages suspended at mid depth, compact mesh cages maintaining clams jammed together, a standard flat cage allowing clams to lay on their side, and ring supports to maintin clams in an upright position. The latter deployment method was selected as our standard deployment treatment for clams undergoing transport and processing tests. Six clams were placed in each containment apparatus and these were maintain in the Niagara River for 21 days.

PROCESSING EXPERIMENT:
After a three week exposure period all clams were retrieved and shucked using clean hexane rinsed stainless steel knives. Clam tissues were drained of excess fluid wrapped in hexane-rinsed aluminum foil and frozen on dry ice. Tissues were kept frozen at -20 °C until analyzed. During this time several processing expriments were undertaken before tissues were frozen. Live clams were held on ice for 2, 8, and 24 hours before shucking and others were held at ambient temperatures for 8 hours. A further group was also shucked and kept on ice for 8 hours before freezing. All others were shucked within 45 minutes of their retrieval.

Laboratory Analysis

Clam tissue analysis of PCB's was done according to Ontario Ministry of the Environment (1983) protocol with a few modifications.

Clam tissues were thawed, weighed and placed in 50 ml screw top (teflon lined) centrifuge tube with 20 ml of concentrated HCl and aggitated for 1.5 - 2 hours. Extraction was done with a 20 ml portion of 25% dichloromethane in hexane (v/v) and aggitated for 1.5 hours. Approximately 0.25 ml of 2-propanol was added to the centrifuge tubes to break the emulsion layer then centrifuged for 20 minutes @ 2000 rpm. The extraction procedure was repeated twice. Sodium bicarbonate followed by sodium thiosulphate was added to the extracts to neutralize and dry samples respectively. Extracts were diluted to 100 ml with hexane and an aliquot representing 1 g of tissue used for cleanup with Florisil 100-200 mesh (dry pack). The remainder of the extract was used for gravimetric lipid determination. Final PCB extracts were reduced to 1 ml using a rotary evaporator, made to 3 ml with 2,2,4-trimenthlypentane (iso-octane) and submitted for analysis. To increase sensitivity extracts of the temperature experiments were reduced to dryness before being submitted for analysis.

All PCB analyses were performed by gas chromatography using electron capture detection (GC/ECD). A Hewlett Packard 5880A Series GC, equipped with a 30m x 0.32 m I.D. SPB-35 capillary column (Supelco, Inc) was used. Typical GC conditions were; 80°C isothermal for 0.50 minutes; then 5°C/minute to 300°C, held at 300°C for 40 minutes. The splitless injection system (1.00 minute valve time) was maintained at 200°C.

Levels of PCB's in the samples were determined by comparison to area response factors obtained for standard solutions of Aroclor 1260 and Aroclor 1254, injected separately.

PCB recovery from controls was 99.8 % ± 13.3 (mean of n=12 ± S.D.). Method detection limit was 8.9 ppb. No corrections were made for recovery efficiencies

Statistical Analysis

Data from the temperature experiments were analyzed as a 5 x 2 factorial ANOVA with replication. Paired comparisons between acclimated and non-acclimated clams at the same temperature were done using the least significant difference test.

In all other experiments ANOVAs followed by Duncan's new multiple-range test were used to test for significant differences.

All statistical analyses were completed as in Steel & Torrie (1980).

RESULTS & DISCUSSION

General Findings

Lipid content of clams ranged from 0.20% to 1.78% with a mean of 0.82% ± 0.039 (mean of n= 69 ± SD). Kauss and Hamdy (1985) found similar lipid levels in *E. complanata* of 0.7 to 1.9%.

Due to the range of lipid levels amoung clams, PCB levels were lipid corrected. This was done by dividing PCB concentration (ng/g tissue) on a wet weight basis by % lipid as a decimal fraction.

PCB levels were inversely correlated with lipid content in clams from all but one experiment. Transport, deployment, and temperature-acclimated experiments were highly correlated ($r=$ -0.91, $r=$ -0.96, & $r=$ -0.88 respectively), while clams from the temperature-nonacclimated experiment had PCB levels only marginally correlated to lipids ($r=$ -0.58). No such relationship was observed in the processing experiment ($r=$ -0.07). This phenomenon has also been observed in birds by Stickel (1973). He attributed this to the retention of fat soluble contaminants even during fat metabolism.

Wet weights of clam tissue ranged from 3.75 to 7.39 grams with a mean of 5.42 ± 0.10 grams (mean of n = 69 ± SD)

Transportation Experiment

Clams transported in a moist state (ATM & ITM) contained marginally higher levels of PCB's than those transported in a wet state (ATW & LTW) although the levels were not significantly greater (= 0.05). See Table 1.

Generally it would seem that the method of transporting clams to the monitoring site is not of significant importance.

Table 1. Mean PCB concentrations and standard deviations of clams in Transport experiment. n = 3.
ATM = ambient temperature moist, ATW = ambient temperature wet, ITM = ice temperature moist, LTW = lake temperature wet, SR = support rings.

TREATMENT	PCB (ng)	LIPID (%)	WET WEIGHT (g)
ATM-SR	41 ± 26	0.57 ± 0.054	5.93 ± 0.15
ATW-SR	17 ± 7.6	1.1 ± 0.053	6.56 ± 0.14
ITM-SR	31 ± 14	0.84 ± 0.044	6.32 ± 0.60
LTW-SR	21 ± 10	0.87 ± 0.041	5.85 ± 0.21

Deployment Experiment

Clams deployed in sand boxes had significantly higher levels of PCB's than those deployed by other methods (= 0.05). Levels were approximately three times higher than clams in standard cages (Table 2.) which are presently in common use. Since PCB's are normally adsorbed to particles, bivalves have to actively siphon to accumulate PCB's (Risebrough et al. 1976). This may indicate that the clams are siphoning water more actively, due to a more natural orientation in the sand box.

TABLE 2. Mean PCB concentrations and standard deviations of clams in Deployment experiment. n = 3.
LTW =lake temperature wet, SR =support ring, CC =compact cage, FC =floating cage, SC =standard cage, SB =sand box.

TREATMENT	PCB (ng)	LIPID (%)	WET WEIGHT (g)
LTW-SR	21 ± 10	0.87 ± 0.041	5.85 ± 0.21
LTW-CC	27 ± 9.5	0.74 ± 0.12	5.24 ± 0.22
LTW-FC	54 ± 12	0.74 ± 0.034	6.04 ± 0.56
LTW-SC	62 ± 22	0.78 ± 0.047	5.01 ± 0.30
LTW-SB	164 ± 31.1	0.24 ± 0.030	6.06 ± 0.18

Processing Experiment

No significant difference (= 0.05) was observed in PCB levels in the five processing treatments tested (Table 3.) indicating that it may not be critical if field logistics do not permit immediate processing of clams.

Table 3. Mean PCB concentrations and standard deviations of clams in the Processing experiment. n = 3. LIH2 = live ice hold for two hours, LIH8 = live ice hold for eight hours, LIH24 = live ice hold for 24 hours, SIH8 = shucked ice hold for eight hours, LAH8 = live ambient hold for eight hours, NH = no hold.

TREATMENT	PCB (ng)	LIPID (%)	WET WEIGHT (g)
LIH2	43 ± 17	0.92 ± 0.25	5.15 ± 0.60
LIH8	27 ± 15	0.64 ± 0.023	5.41 ± 0.15
LIH24	29 ± 2.8	0.78 ± 0.28	5.40 ± 0.29
SIH8	33 ± 3.9	1.2 ± 0.082	5.74 ± 0.45
LAH8	31 ± 2.4	1.0 ± 0.082	4.85 ± 0.41
NH	21 ± 10	0.87 ± 0.041	5.85 ± 0.21

Temperature Experiment

Temperature did not have a significant (= 0.05) affect on PCB accumulation on either acclimated (Table 4.) or non-acclimated clams (Table 5.). Acclimated clams however had PCB levels two to four times higher than non-acclimated clams. Only at 20°C was this difference significant (= 0.05) however.

Table 4. Mean PCB concentrations and standard deviations of acclimated clams in the Temperature experiment. n = 3.

TEMP (°C)	PCB (ng)	LIPID (%)	WET WEIGHT (g)
5	172 ± 25.3	0.51 ± 0.020	5.00 ± 0.050
10	219 ± 32.3	0.62 ± 0.032	5.31 ± 0.19
15	146 ± 13.6	0.54 ± 0.17	5.25 ± 0.32
20	220 ± 102	0.41 ± 0.12	4.64 ± 0.15
25	28 ± 3.3	1.00 ± 0.14	4.52 ± 0.34

Table 5. Mean PCB concentrations and standard deviations of non-acclimated clams in Temperature experiment. n = 3.

TEMP (°C)	PCB (ng)	LIPID (%)	WET WEIGHT (g)
5	41 ± 19	1.00 ± 0.030	5.48 ± 0.26
10	92 ± 5.6	0.97 ± 0.098	5.71 ± 0.43
15	34 ± 14	1.11 ± 0.054	6.16 ± 0.26
20	63 ± 15	1.01 ± 0.070	4.93 ± 0.50
25	103 ± 12.8	1.78 ± 0.343	4.94 ± 0.44

Conclusions

1) Clams can be transported in the field under more practical conditions than has been previously assumed. For example clams may be kept moist rather than submerged in water and be subjected to ambient temperature fluctuations of 15 - 20 °C without affecting PCB bioaccumulation rates.

2) Greater latitude can also be taken in the field with respect to holding clams before freezing. Keeping them on ice for 24 hours, shucking and holding for 8 hours, and holding at ambient temperatures for 8 hours before freezing result in the same level of PCB accumulation as clams that are processed within 45 minutes of recovery.

3) The level of PCB's in indigenous clams may not be directly comparable to levels accumulated in clams introduced to a site in cages. Clams deployed in sand boxes accumulated PCB's to a level three times greater than any of the other clam cage experiments including the standard flat cage.

4) Varying cage configurations are not likely to be a source of error when comparing data from different studies. All experimental mesh cages, (flat; compact; floating), as well as the support rings used in this study produced statistically identical results with respect to PCB's bioaccumulation.

5) The seasonal range of monitoring programs may be expanded. Temperature within the 5 - 25 °C range did not have a significant affect on PCB accumulation on either acclimated or non-acclimated clams.

6) Acclimation procedures may actually stress clams and cause their lipid levels to be reduced. Acclimated claams accumulated PCB levels 2 to 4 times higher than non acclimated clams. This was significant at 20 °C.

7) The dynamic interactions involving PCB bioaccumulation, lipid metabolism and physiological responses of clams to stress need to be studied in greater detail in order to improve the resolution of clam bioaccumulation monitoring techniques.

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B8

BIOMONITORING : CHEMICAL DEPENDENT QUANTITATIVE RELATIONSHIPS FOR THE BODY BURDENS OF ORGANIC CHEMICALS IN AQUATIC ORGANISMS

Frank, A.P.C. Gobas, Ronald, W. Russell and G.D. Haffner
The Great Lakes Institute
University of Windsor
Windsor, Ontario, N9B 3P4

Introduction

One of the most important issues presently addressed in aquatic toxicology is the interpretation of aqueous concentrations of toxic organic chemicals in terms of exposure and effects to aquatic life. Direct measurement of quantities of trace organic chemicals in the water is not only difficult but it is also insufficient to determine exposure and effects to aquatic organism. Since many organic chemicals were observed to accumulate in aquatic organisms, resulting in concentrations in the organism exceeding those in the water by orders of magnitude, it was suggested to use organisms to monitor chemical concentrations in the water and to determine chemical exposure to aquatic life. Organisms used for this purpose such as mussels and various species of fish are generally referred to as biomonitors. The chemical concentration in the biomonitor is therefore viewed as a measure of the chemical concentration in the water and the exposure of the organism to this chemical. But in order to determine chemical concentrations in water from the body burden of the biomonitor the relationship between the chemical concentrations in the water and the organism has to be established. This relationship reflects the organism's ability to absorb chemical from the water, its food and other sources and to depurate the chemical. In this paper we will review the mechanism of chemical uptake and depuration of organic chemicals in aquatic organisms and the kinetics of chemical uptake and elimination will be discussed. Finally, we will present chemical dependent and organism specific relationships relating chemical concentrations in aquatic organisms to chemical concentrations in the water and show how these relationships should be used to set deployment schemes for biomonitors and to interpret biomonitoring data.

Uptake, depuration and bioaccumulation in aquatic organisms

The expression describing the simultaneous uptake of chemical from food and water in aquatic organisms as well as the depuration of that chemical to the water (via the gills), into the faeces and by metabolic transformation can be expressed as

$$dC_F/dt = k_1 . C_W - k_2 . C_F + k_A . C_A - k_E . C_F - k_R . C_F \qquad (1)$$

where C is concentration (mol/m^3), t is time (h), and the subscripts W refer to water, A to food, E to faeces, and F to the whole organism (Gobas et al. 1988, 1989). The organism is defined as the whole organism excluding the gill compartment and the gastro-intestinal (GI) tract. k_1, k_2, k_A and k_E are respectively the rate constants (h^{-1}) of chemical uptake from the water, elimination to the water, uptake from food, and elimination by egestion in the faeces. k_R is the rate constant (h^{-1}) for metabolic transformation of the chemical in the organism.

Following the fugacity approach, discussed at great detail by Mackay and coworkers (Mackay and Paterson 1982), equation 1 can also be written as

$$V_F.Z_F.df_F/dt = D_F.(f_W - f_F) + D_A.f_A - D_E.f_F - D_R.f_F \quad (2)$$

where V is volume (m^3), Z is the chemical's fugacity capacity ($mol/m^3.Pa$) in a phase, and f is the chemical's fugacity (Pa). D_F is the overall transport parameter (mol/Pa.h) for chemical transfer between water and fish across the respiratory surface (e.g. gills). D_A is the transport parameter for chemical uptake from food into the organism across the gastro-intestinal (GI)-tract. The transport parameter D_E (mol/Pa.h) describes chemical elimination in the faeces. D_R (mol/Pa.h) is the transformation parameter for metabolic transformation of chemical in the organism. The transport parameters D_F, D_A, and D_E include all transport processes involved in solute transfer between the water, food, and faeces, respectively, and the solute's final storage site in the fish.
Integration of equation 1 with a constant C_W and C_A, an initial

C_F of zero and assuming a constant fish volume with time gives

$$C_F = \{C_W.[k_1/(k_2+k_E+k_R)] + C_A.[k_A/(k_2+k_E+k_R)]\}.\{1-\exp-(k_2+k_E+k_R).t\} \qquad (3)$$

This equation describes the chemical concentration in the organism with time when an uncontaminated organism, with no initial chemical concentration, is exposed to water with a constant concentration of the chemical for a certain length of time.

Since C_F is f_F/Z_F, C_W is f_W/Z_W and C_A is f_A/Z_A, it can be shown that equation 3 is equivalent to the integrated form of equation 2 with a constant f_A and f_W and an initial f_F of zero, i.e.,

$$f_F = \{f_W.[D_F/(D_F + D_E + D_R)] + f_A.[D_A/(D_F + D_E + D_R)]\}.\{1 -\exp[-(D_F + D_E + D_R).t/(V_F.Z_F)]\} \qquad (4)$$

The fugacity and the kinetic description of the bioconcentration process are similar in that they are based on the same assumptions, namely that (i) chemical transfer is passive and (ii) the organism is viewed as one, homogeneous compartment. The difference in the two approaches is that in the kinetic approach bioaccumulation is viewed as a balance between the rates of chemical uptake and elimination, whereas in the fugacity approach bioaccumulation is viewed as a process where the chemical is attempting (but not necessarily achieving) to reach a thermodynamic equilibrium. This thermodynamic equilibrium is characterized by equal fugacities of the chemical in the organism, the water and the food consumed by the organism. The strength of the kinetic descriptions is that the rate constants can be measured directly from uptake and depuration experiments. The fugacity-equations, however, distinguish between thermodynamically controlled partitioning phenomena, characterized by the fugacity capacity values (i.e. Z) and pure transport phenomena, described by transport parameters (i.e. D). Fugacity expressions therefore often give an in-depth view of the actual mechanism of the bioaccumulation process. The two approaches complement each other, and are best combined. This can be easily achieved by comparing equations 3 and 4, from which it follows that

$$k_1 = D_F/V_F.Z_W \tag{5}$$

$$k_2 = D_F/V_F.Z_F \tag{6}$$

$$k_A = D_A/V_F.Z_A \tag{7}$$

$$k_E = D_E/V_F.Z_F \tag{8}$$

$$k_R = D_R/V_F.Z_F \tag{9}$$

Equations 3 and 4 show that at infinite exposure time an organism-water bioaccumulation factor, K_B can be defined for an organism simultaneously exposed to contaminated water and food as

$$K_B = C_F/C_W = (Z_F/Z_W).\{[D_F/(D_F+D_E+D_R)] + [(f_A/f_W).(D_A/(D_F+D_E+D_R)]\}$$

$$(10)$$

or

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R)\} \quad (11)$$

It also follows from equations 3 and 4 that in food uptake experiments, when organisms are exposed to contaminated food but uncontaminated water, the ratio of organism to food concentrations, i.e., C_F/C_A or K_M, which is the biomagnification factor, can be expressed as

$$K_M = C_F/C_A = (Z_F/Z_A).\{D_A/(D_F+D_E+D_R)\} = k_A/(k_2+k_E+k_R) \quad (12)$$

The bioconcentration factor, K_C, which is defined as the ratio of fish and water concentrations at infinite exposure time for organisms exposed to contaminated water only (i.e., $C_A = f_A = 0$), is

$$K_C = C_F/C_W = (Z_F/Z_W).\{D_F/(D_F + D_E + D_R)\} \quad (13)$$

Equations 11 to 13 demonstrate that K_B, K_M, and K_C are not solely determined by the thermodynamic quantities, i.e., Z_F, Z_W and Z_A, which reflect the affinities of the chemical for the organism, water, and food, but also by the relative rates of chemical uptake from water and food, release to the water, egestion with the faeces, and metabolic transformation. Equation 13 shows that even when no metabolic transformation occurs ($D_R = 0$), the

bioconcentration factor only reflects organism-water partitioning when D_E is small compared to D_F.
It thus follows that in order to make reliable predictions about the bioaccumulation potential of hydrophobic chemicals in aquatic organisms and the rate at which bioaccumulation is achieved in the organisms, knowledge is required about the processes controlling the exchange of solute between fish, water, food, and faeces.

Equation 3 illustrates that when a contaminated organism is introduced in clean, uncontaminated water (C_W is zero) and consumes uncontaminated food (C_A is zero), it will lose chemicals to the water resulting in a drop of C_F with time. The differential equation describing this process is again equation 1, but with a C_W and C_A of zero, i.e.

$$dC_F/dt = -(k_2 + k_E + k_R).C_F \qquad (14)$$

which after integration with an initial $C_{F,t=0}$ becomes

$$C_F = C_{F,t=0}.\{\exp(-(k_2 + k_E + k_R).t)\} \qquad (15)$$

or

$$\ln C_F = \ln C_{F,t=0} - (k_2 + k_E + k_R).t \qquad (16)$$

Equation 16 demonstrates that in a logarithmic plot ln C_F decreases linearly with time. The slope of this plot is the total depuration rate constant $(k_2 + k_E + k_R)$ and has units of reciprocal time.

The rate at which chemicals are eliminated by organisms can also be expressed by the biological half-time in the fish. This is the time to reach half the initial concentration in the fish for an organism in uncontaminated water. It follows from equation 16 that the biological half-time, $t_{1/2}$ is

$$t_{1/2} = \ln 2/(k_2 + k_E + k_R) = 0.693/(k_2 + k_E + k_R) \qquad (17)$$

The time required to achieve steady state, i.e. a constant ratio between the chemical concentrations in the organism and the water or K_B, is also dependent on the rate constants for chemical depuration. It can be shown that the time required to reach 95% of the steady state t_{95} is

$$t_{95} = - \ln 0.05/(k_2 + k_E + k_R) = 3.0/(k_2 + k_E + k_R) \qquad (18)$$

This time t_{95} is of crucial importance in biomonitoring since it determines how long the biomonitor should be employed before the concentration in the organism reflects the concentration in the water through the bioaccumulation factor. This time is chemical specific since the rate constants k_2, k_E and k_R are chemical dependent. We will now discuss the kinetics of chemical uptake in aquatic organism and demonstrate the chemical dependence of the uptake and depuration rate constants.

Chemical and organism specific relationships for the uptake and depuration of organic chemicals in aquatic organisms

Lipid-water mass transfer models were derived by Gobas and Mackay (1987) and Mackay and Hughes (1984) to gain further insight into the processes controlling the exchange of chemical between aquatic organisms and water and to develop practical procedures to estimate the bioconcentration kinetics of chemicals in fish. The authors used the fugacity approach to derive the model equations but presented their final model in terms of rate constants.

The main feature of this model is that it views the exchange of solute chemical between the water and the organism to take place in a series of aqueous and lipid layers. All transport processes in water phases are therefore grouped together in one overall water phase transport parameter D_W. This overall water phase transport parameter contains all transport parameters $D_{W,i}$ in water phases. The transport parameters $D_{W,i}$ can refer to diffusion, in which case $D_{W,i}$ equals $k.A.Z_W$, where k is the mass transfer coefficient (m/s), A is area of diffusion and Z_W is the chemical's fugacity capacity in the water phase. It can also refer to non-diffusive transport, where the solute is conveyed by a fluid flow G (m^3/s) such that $D_{W,i}$ equals $G.Z_W$. The overall transport parameter D_W can therefore also be expressed as $Q_W.Z_W$, where the transport parameter Q_W (m^3/s) combines all k.A and flow rates G in water phases of the organism. The transport parameter

Q_W is therefore sometimes viewed as an "hypothetical water flow rate" in the organism.

Similar to D_W all transport processes in lipid phases are combined in an overall lipid phase transport parameter D_L. This lipid phase parameter D_L can be designated $Q_L.Z_L$, where Q_L combines all k.A and flow rates G in lipid phases of the organism and can be considered the "hypothetical lipid flow rate" in the organism. The transport parameters D_W and D_L therefore refer to the net transport of chemical in respectively the water and the lipid phase of the organism.

Assuming that the water and lipid transport processes apply in series it follows that the transport parameter for chemical exchange between the water and the organism D_F can be expressed as

$$1/D_F = 1/D_W + 1/D_L = 1/Q_W.Z_W + 1/Q_L.Z_L \qquad (19)$$

Since D_F equals $k_1.V_F.Z_W$ or $k_2.V_F.Z_F$ (i.e. equations 5 and 6) and since bioaccumulation is predominantly in the lipids of the organisms Z_F can be expressed as a fraction L_F of the fugacity capacity in the lipids i.e. as $L_F.Z_L$, where L_F is the lipid fraction of the organism (i.e. V_L/V_F), equation 19 can be rewritten as

$$1/k_2 = V_L.\{(Z_L/Q_W.Z_W) + 1/Q_L\} \qquad (20)$$

$$1/k_1 = V_L.\{1/Q_W + (Z_W/Q_L.Z_L)\}/L_F \qquad (21)$$

When the lipid-water partition coefficient Z_L/Z_W or K_L is replaced by the 1-octanol-water partition coefficient Z_O/Z_W or K_{OW} (thus assuming Z_L to be equal to Z_O) equations 20 and 21 become

$$1/k_2 = V_L \cdot \{(K_{OW}/Q_W \cdot Z_W) + 1/Q_L\} \qquad (22)$$

$$1/k_1 = V_L \cdot \{1/Q_W + (1/Q_L \cdot K_{OW})\}/L_F \qquad (23)$$

The ratios V_L/Q_L and V_L/Q_W can be viewed as the times of chemical transport in V_L m^3 of respectively lipids and water. However, if transport of a given amount of chemical requires a volume V of lipid, it will require a much larger volume i.e. $K_{OW} \cdot V_L$ of water, since the chemical concentration in the water is a factor of K_{OW} lower than in the lipids. The time for the water phase in the organism to transport a certain amount of chemical is therefore K_{OW} times longer than that for the lipid phase. The transport time of the water phase is therefore multiplied with K_{OW} in equation 22 and alternatively the lipid transport time is divided by K_{OW} in equation 23. Since the lipid and water transport processes occur in series these times are additive and the longer time "controls" the bioconcentration kinetics.

The expressions 22 and 23 contain two types of variables namely, (i) biological parameters i.e. V_L, Q_W, Q_L, G_V and L_F, which are specific to a particular fish and its physiological condition and

(ii) a chemical parameter K_{OW} expressing the chemical's tendency to partition between lipids and water.

When for a series of chemicals, varying in K_{OW}, rate constants or uptake efficiencies have been determined in a particular organism equations 22 and 23 can be fitted to the experimental data resulting in values for Q_W and Q_L. The data fitting involves a regression e.g. $1/E_0$ versus $1/K_{OW}$ or $1/k_2$ versus K_{OW}. Since K_{OW} usually varies over several orders of magnitude and would thus weigh heavily in favour of the data points with high K_{OW} simple linear regression (e.g. $1/E_0$ versus $1/K_{OW}$) leads to erroneous results. Instead regression should be performed on a semi-logarithmic basis e.g. $1/E_0$ versus log K_{OW} and $1/k_2$ versus log K_{OW} but using the linear equations 22 and 23. Fitting these equations to experimental data is therefore best performed by using non-linear regression techniques.

Gobas and Mackay (1987) and Gobas et al. (1989) fitted equations 22 and 23 successfully to kinetic data in various fish species i.e. the rainbow trout, goldfish and guppy resulting in values for Q_W and Q_L. It was also found that Q_W increases with increasing body weight of the organism as

$$Q_W = 1.4.W^{0.6} \qquad (24)$$

Although conclusive data are presently lacking there seems to be a similar relationship for Q_L i.e.

$$Q_L = 0.014.W^{0.6} \qquad (25)$$

Theoretical justification for the empirical relationship between Q_W and M can be obtained from studies on the influence of oxygen concentration on gill ventilation rates. Norstrom et al. (1976) showed that the gill ventilation volumetric rate G_V is a function of the oxygen concentration in the water C_{OX} (ml of O_2/ml of water), the oxygen transfer efficiency across the gills E_{OX}, the metabolic rate coefficient (ml of $O_2.h^{-1}.g^{-0.8}$) and the body weight of the organism to the power 0.8, i.e.

$$G_V = A.M^{0.8}/(E_{OX}.C_{OX}) \qquad (26)$$

The similarity in body weight dependence of the gill ventilation volumetric rate and Q_W suggest a similarity in these two quantities. More importantly, the similarity in the actual values of Q_W and directly measured or estimated (i.e. equation 26) gill ventilation rates indicates that Q_W is dominated by the gill ventilation volumetric rate.

A similar lipid-water mass transfer model can also be developed for chemical uptake from consumed food and elimination to faecal matter in the GI-tract. The derivation of this model can be found in Gobas et al. 1988. It was shown that similar to equations 22 and 23 chemical specific relationships can be derived for the rate constants k_A and k_E and the chemical uptake efficiency from food E_{FO} i.e.

$$1/k_A = (V_F/G_I).\{(G_O.L_G/Q_{WF}).K_{OW} + (G_O.L_G/Q_{LF}) + 1\} \quad (27)$$

$$1/k_E = (V_F.L_F/G_O.L_G).\{(G_O.L_G/Q_{WF}).K_{OW}+(G_O.L_G/Q_{LF})+1\} \quad (28)$$

$$1/E_{fO} = (G_O.L_G/Q_{WF}).K_{OW} + G_O.L_G/Q_{LF} + 1 \quad (29)$$

where G_I is the volumetric feeding rate (in m^3 food per hour), G_O is the volumetric egestion rate (in m^3 faeces per hour), Q_{WF} is the water phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour), Q_{LF} is the lipid phase transport parameter for chemical exchange between the GI-tract and the final storage site in the organism (in m^3 per hour) and L_G is the organic or "lipid" fraction of the gastro-intestinal contents (in grams of organic matter per gram of GI contents).

The reciprocal of k_A, i.e., $1/k_A$, can be viewed as the time needed to transport chemical from the food into the fish or as the total resistance for chemical transfer from the food into the fish. Likewise, $1/k_E$ is the time required to eliminate chemical from the fish into the faeces or the total resistance for chemical elimination to the faeces. The ratios $(V_F/G_I).(G_O.L_G/Q_W).K_{OW}$ and $(V_F/G_I).(G_O.L_G/Q_L)$ can be viewed as the solute's relative transport times in the water and lipid phases, respectively, of the fish or as the relative resistances that the solute encounters in the water and lipid phases of the fish on its route from the food phase in the GI-tract to the final storage site in the body lipid of the fish. When the solute's K_{OW} increases, and aqueous solubility thus decreases, the water phase of the fish can accommodate only a lower concentration of solute molecules. As a result, the time required to transport a certain amount of solute with this lower concentration increases. The resistance of the fish's water phase toward mass transfer thus increases, whereas it remains approximately constant in the lipid phase. For high K_{OW} chemicals, this implies that the uptake rate from food and elimination rate to the faeces and thus k_A, E_{fO} and k_E decrease with increasing K_{OW}. For low K_{OW} chemicals, uptake from food and elimination by excretion to the faeces is predominantly controlled by transport in lipid phases, and k_A, E_{fO}, and k_E are, therefore, expected to be approximately constant with respect to K_{OW}.

Equation 29 demonstrates that by experimental measurement of E_{fO} for a series of chemicals with varying K_{OW} under controlled conditions, i.e., a constant feeding rate and no uptake of chemical from the water, it is thus possible to determine the fundamental kinetic parameters Q_W and Q_L. Knowledge of these parameters is invaluable for reliable estimation of organic chemical bioaccumulation from contaminated food.

Gobas et al. (1988) showed that experimental data for dietary uptake of chemicals in fish fit this simple relationship with values for $(G_O.L_G/Q_W).K_{OW}$ of 5.3 (+/- 1.5).10^{-8} and for $(G_O.L_G/Q_L + 1)$ of 2.3 (+/- 0.3), thus resulting in the following relationship for E_{fO}, k_A and k_E

$$1/E_{fO} = 5.3.10^{-8} + 2.3 \qquad (30)$$

$$1/k_A = (V_F/G_I).(5.3.10^{-8} + 2.3) \qquad (31)$$

$$1/k_E = (V_F.L_F/G_O.L_G).(5.3.10^{-8} + 2.3) \qquad (32)$$

The relationships that have been presented above for the chemical uptake and elimination kinetics have been experimentally validated in several species of fish (Gobas and Mackay 1987, Gobas et al. 1988 & 1989). However, for other aquatic biomonitors such as the mussel or mayfly the above relationships for the uptake and elimination rate constant have yet not been established because of a lack of experimental data. We believe that when these experimental data become available similar relationships can be derived for biomonitors other than fish.

The use of kinetic rate constants in biomonitoring

We will now demonstrate with an illustrative example how kinetic rate constants should be used to set deployment schemes for aquatic biomonitors and to translate chemical concentrations in the biomonitor to concentrations in the water. Before a biomonitor can be used as a tool to measure chemical concentrations in the water the chemical uptake and elimination kinetics should be established. Preferably, this is achieved by performing uptake and elimination experiments in the field, which is often very difficult, or in the laboratory. These experiments should involve the chemicals that will be monitored. This procedure, which can be viewed as the "calibration" of the biomonitor, will establish the rate constants for uptake and elimination of each chemical of interest. If it is impossible to perform this "calibration" procedure the rate constants can be derived with the expressions discussed earlier as we will now demonstrate with an illustrative example. For this purpose we will use a 5 gram fathead minnow (V_F is 0.005 L)with a lipid content of 6 %, as a biomonitor. The biomonitor will be deployed in a cage to monitor chemical exposure from the water. We will assume that the fish is contaminant free at the time of deployment. To derive the rate constants for chemical uptake from and elimination to the water i.e. k_1 and k_2 equations 22 to 25 can be used. Equations 24 and 25 show that the Q_W and Q_L for the fathead minnow are respectively $1.4.5^{0.6}$ i.e. 3.7 L/d and $0.014.5^{0.6}$ i.e. 0.037 L/d. Substitution of Q_W and Q_L in equations 22 and 23 then results in

$$1/k_1 = 0.00136 + 0.136/K_{OW} \qquad (33)$$

$$1/k_2 = 8.1.10^{-5}.K_{OW} + 0.0081 \qquad (34)$$

Equations 33 and 34 demonstrate that for trichlorobenzene (TCB) with a log K_{OW} of 4.0, k_1 is 728 d^{-1} and k_2 is 1.2 d^{-1}. For mirex with a log K_{OW} of 7.5 these rate constants are respectively 735 d^{-1} and 0.0004 d^{-1}.

The rate constants for chemical uptake from food and elimination to the faeces i.e. k_A and k_E can be derived from equations 31 and 32. Assuming that the caged fathead minnow feeds at a feeding rate of 1 % of its own body weight per day, k_A for TCB is 0.0043 d^{-1} and for mirex is 0.0025 d^{-1}. Assuming that the faecal egestion rate G_O is one-third of the feeding G_I and L_F and L_G are approximately equal equation 32 shows that k_E can be calculated to give values of 0.0014 d^{-1} for TCB and 0.00083 d^{-1} for mirex. When we assume no metabolic transformation of TCB and mirex in the fish the total depuration rate constant k_T (i.e. the sum of k_2, k_E and k_R) can be determined as (1.2+0.0014) i.e. 1.2014 d^{-1} for TCB and (0.0004 + 0.00083) i.e. 0.00123 for mirex. These calculations demonstrate that for TCB chemical elimination is predominantly to the water and that chemical elimination to the faeces is unimportant. For mirex the situation is quite different with 2/3 of its body burden being eliminated in faecal matter and only 1/3 to the water.

Comparison of the uptake rate constants k_1 and k_A shows that for TCB chemical uptake from the food will become a significant route of exposure when the chemical concentration in the food C_A is approximately 150,000 times higher than that in the water. In that case the chemical uptake rate from the water $k_1.C_W$ would equal the uptake rate from the food $k_A.C_A$ i.e. water and food are equally important exposure routes. For a chemical with a K_{OW} of only 10,000 it is extremely unlikely to have concentrations in food sources that exceed the chemical concentration in the water by that much. A reasonable estimate of the chemical concentration in the food can be derived by assuming a chemical equilibrium of the chemical in the food with that in the water i.e. C_A equals $0.05.K_{OW}.C_W$ (Mackay 1982). For TCB this implies a C_A, which is only 500 times larger than C_W. It can thus be concluded that for TCB gill ventilation of water is the main exposure route. The body burden of the biomonitor thus reflects the concentration in the water. The relationship between the concentration in the organism and the water can now be established by substituting the calculated (or measured) values for k_1, k_2, k_E, k_A and k_R in equation 11 i.e.

$$K_B = C_F/C_W = \{728/(1.2+0.0014+0)\} + \{0.05.10^4.(0.0043/(1.2+0.0014+0))\} = 607 \qquad (35)$$

It thus follows from equation 35 that C_W equals the measured concentration in the biomonitor divided by 607 i.e. $C_F/607$. However, it should be noted that this procedure is only valid when the organism and water are at steady-state at the time of sampling. In practice, this steady state will be practically reached after $3.0/(k_2 + k_E + k_R)$ i.e. 2.5 days assuming a chemical concentration which does not vary in time. The fish should thus be employed for at least 3 days before the simple correlation of $C_F/607$ can be used to derive the chemical concentration in the water.

For mirex the situation is again quite different. With values for k_1 and k_A of respectively 735 d^{-1} and 0.0025 d^{-1} it follows that the chemical concentration in the food of the organism has to be approximately 300,000 fold higher than the concentration in the water before food and water are equally important exposure routes for the fish. However, for a chemical with a K_{OW} of 32,000,000 simple partitioning of the chemical in the food source of the organism may cause such a large difference in chemical concentrations in the water and food. Assuming equilibrium partitioning of mirex between the food and the water results in an estimate of C_A, which is $0.05.K_{OW}$ i.e. 1,600,000 fold higher than C_W. In that case 84% of the total chemical exposure is chemical uptake from food and only 16% of the body burden of the organism is chemical taken up from the water. It thus follows that chemical uptake from food is the main exposure route for mirex in the fish.

The body burden of mirex in the fish therefore most closely reflects the concentration in the food source, not the water.

If the fish is used to monitor the chemical concentration in the water or the food, it is necessary to know either the chemical concentration in the water or in the food or the relationship between the chemical concentrations in the food and water. Since the main purpose of the biomonitor is to "measure" the chemical concentration in environmental compartments such as the water and food, this seems to contradict the purpose of the biomonitor. However, the body burden C_F for mirex is the function of two parameters i.e. C_A and C_W. Different combinations of the parameters C_W and C_A can therefore result in the same C_F. In order to translate body burdens into concentrations in the water it is necessary to know C_A or to make an estimate about the value for C_A. As discussed earlier, $0.05.K_{OW}.C_W$ is often a reasonable estimate for C_A. It then follows that K_B can be calculated as

$$\begin{aligned} KB = \; & C_F/C_W = \{735/(0.0004 + 0.00083 + 0)\} + \\ & \{0.05.10^{7.5}.(0.0025/(0.0004 + 0.00083 + 0))\} \qquad (36) \end{aligned}$$

Equation 36 thus shows that C_W can be calculated as $C_F/9.2.10^5$. The time required to reach this steady state is condition is $3.0/(k_2 + k_E + k_R)$ i.e. 2440 days. This shows that for mirex the biomonitor has to be deployed for a much longer time than when TCB is being monitored. It may even be possible that the biomonitor will never reach this steady state condition within its life time. The body burden for mirex in the biomonitor is thus a function of the deployment time t. This time function is given by equation 3, which after substitution of the values of the rate constants is

$$C_F = \{735.C_W/(0.0004 + 0.00083 + 0)\} + \{0.05.10^{7.5}.C_W.(0.0025/(0.0004 + 0.00083 + 0))\} \quad (37)$$

The example discussed above shows the necessity to interpret biomonitoring data on a chemical specific basis. It can thus be concluded that when biomonitors are to be used as a tool to measure chemical concentrations in the water and chemical exposure the kinetics of chemical uptake and depuration have to established for each chemical of interest.

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BIOMONITORING PROTOCOLS FOR ADULT AQUATIC INSECTS: CONTAMINANT TRENDS, SAMPLE SIZE AND SENSITIVITY

Zsolt E. Kovats and Jan J.H. Ciborowski
Department of Biological Sciences
University of Windsor
Windsor, Ontario, N9B 3P4

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ABSTRACT

Benthic aquatic insect larvae living in contaminated sediments accumulate significant organochlorine burdens. However, their value as indicators is limited by sampling difficulties and the necessity of acquiring enough biomass for analysis. The nocturnal, photophilic, winged adult stages are more easily collected than larvae. Our objectives were to assess seasonal variation in adult insect availability and contaminant burden, contrast concentrations in animals from contaminated sites with those from uncontaminated areas, and determine minimum sample biomass that provides reasonable detection limits for organochlorine contaminants (PCBs, pesticides and others). Light trap collections yielded large samples of Trichoptera (mostly *Cheumatopsyche*) from late May to late August at sites on the Detroit and St. Clair rivers. Ephemeroptera (*Hexagenia*, *Caenis*) were abundant for only 1-2 weeks in midsummer. Midsummer collections yielded many Trichoptera at five sites along the Niagara River. However, low temperatures limited the size of catches of Trichoptera, *Hexagenia* and *Caenis* at four locations on the St. Marys River. Contaminant concentrations in animals from Detroit River samples were typically 1-2 orders of magnitude greater than in animals from several central Ontario control sites. Series of triplicate subsamples of different mass from a single large collection were analysed for 30 organochlorine compounds. The proportion of contaminants at detectable concentrations and median coefficient of variation stabilized in samples ≥ 0.4 and ≥ 0.8 g dry mass for animals from contaminated sites and uncontaminated sites, respectively. Seasonal variation in contaminant concentration at sites on the Detroit and St. Clair rivers will be compared with spatial variation among major Great Lakes connecting channels.

1.0 INTRODUCTION

Organochlorine contamination of the Laurentian Great Lakes has caused increasing concern in the past two decades. It is now generally accepted that sediment contamination may influence the entire fauna of a water body through uptake by benthic invertebrates and subsequent transfer through the food chain to higher trophic levels (Struger et al. 1985). Consequently, biological monitoring of sediment contaminants has been the focus of much research in recent years.

Degree of contaminant bioaccumulation by aquatic animals depends on the type of exposure and the chemical properties of the compounds (Reynoldson 1987). Benthic invertebrates live on or within the sediments and are exposed to organic contaminants through direct contact and feeding. Freshwater mussels (Kauss and Hamdy 1985), Chironomidae (Diptera) (Larsson 1984), oligochaete worms (Oliver 1984), and caddisfly larvae (Bush et al. 1985) have all been reported to carry high organochlorine contaminant burdens. Since contaminant body burdens are often proportional to concentrations in the sediment (Larsson 1984), benthic invertebrates are potentially useful as indicators of sediment contamination. However, difficulties encountered when sampling bottom-dwelling organisms and the need for extensive processing often limit the amount of tissue available for analyses.

Collection of the adults of benthic insects presents a cost-effective alternative to benthic sampling. Caddisflies (Trichoptera) and mayflies (Ephemeroptera) spend most of their life as larvae, within or in contact with the sediments. The night-active winged adults emerge during the summer in large numbers. Adults are shortlived, do not feed or defecate, and with the exception of a small proportion of contaminants shed with the larval skin (Larsson 1984), body burdens remain unchanged following emergence.

Mauck and Olson (1977), Clements and Kawatski (1984), and Ciborowski and Corkum (1988) analysed adults of aquatic insects collected near large rivers and detected elevated levels of organochlorine contaminants, indicative of sediment concentrations. Though these studies demonstrated the potential for use of these animals as indicators of sediment contamination, there is a need for studies evaluating insect seasonal availability and dispersal, spatial variation in contaminant burden, and minimum useful sample size for contaminant analyses. Our previous work (Kovats et al. 1987) has shown that adult aquatic insects can be collected in large numbers using light traps, and are available in sufficient numbers for analysis throughout the summer. Preliminary results of dispersal studies have shown that active dispersal by adults is limited.

In this paper we compare contaminant concentrations in adult aquatic insects collected at contaminated and uncontaminated sites. We also evaluate analytical precision associated with different sample weights and the effect of differences in storage temperature and length of storage time on analytical results. Such data are necessary if standard techniques for routine monitoring are to be developed.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Adult aquatic insect samples collected from mid-May to mid-September 1987 were analyzed for 30 organochlorine contaminants (19 PCB congeners, 8 pesticides, octachlorostyrene (OCS), hexachlorobenzene (HCB), pentachlorobenzene (QCB)) by gas chromatography (GC). Insects were collected with modified Pennsylvania type light traps (Frost 1957) placed at 4 sites along the Detroit and St. Clair rivers (Table 1) and at three sites in

Table 1. Locations of sampling stations.

Stn	River	Designation	Latitude (North)	Longitude (West)
1	Detroit	River Canard	42°11'48"	83°06'13"
2	Detroit	East Windsor	42°20'27"	82°56'56"
3	St Clair	Sombra	42°42'02"	82°29'03"
4	St Clair	Sarnia	42°54'12"	82°27'29"

central Ontario. Detroit and St. Clair River sediments contain elevated levels of organochlorine contaminants and the rivers have been designated Areas of Concern (International Joint Commission, 1985). Central Ontario sites were chosen to represent uncontaminated areas. Sampling was also conducted along the St. Marys and Niagara rivers in 1988 to evaluate the applicability of our methods accross broader geographic regions (Kovats et al. In prep.).

The catchment reservoir of the light traps contained dry ice, which immobilized insects without introducing contaminants. Detailed collection procedures and specific sample dates were outlined by Kovats et al. (1987). Samples were separated into constituent taxa (Hexagenia (Ephemeroptera), Hydropsychidae (Trichoptera), Other Trichoptera, and Other Taxa) using hexane-rinsed forceps and stored at -20 or -70°C (see below) prior to analysis.

Triplicate 2-5 g samples (fresh weight) of insects were prepared for contaminant analysis. Sample mass used depended on availability. An additional, similar-sized portion was dried at 105°C for 24 h and reweighed to estimate relative moisture. The dry:fresh weight ratio of this sample was used to estimate dry weights of GC-analyzed samples. Replicates from each site were extracted and analyzed on different days. One solvent blank and 5 insect samples were extracted and analyzed on any one day.

2.2 Gas Chromatographic Analysis

Samples were homogenized with mortar and pestle in 50 g Na_2SO_4. Solid-liquid extraction was employed to extract contaminants and lipids (20 g Na_2SO_4 and 300 mL 50% dichloromethane (DCM)-50% hexane). The extract was concentrated to 5 mL by rotary evaporator and the concentrate was added to a Biobeads column (S-X3, 200-400 mesh). Two fractions were eluted by addition of 300 mL 45% DCM-55% hexane mixture. Solvent was evaporated from the first fraction and the remaining lipid residue was weighed. The second fraction containing all extracted organochlorine compounds, was concentrated to 2 mL and cleaned by passage through a column containing 8 g Florisil. Two fractions were eluted by hexane and 50% DCM-50% hexane, respectively. Both were concentrated to 2 mL, diluted with hexane to 10 mL, and 1-µL portions were injected into the GC. Specific conditions and methodology employed during gas chromatography were as outlined by Ciborowski and Corkum (1988). Contaminant concentrations were quantified based on peak patterns by comparison to those on chromatograms of standard mixes of known organochlorine concentrations. Recovery efficiencies were evaluated by spiking uncontaminated samples with known amounts of standard mixes.

2.3 Sample Storage Time and Temperature

The effect of storage temperature was evaluated by analyzing insects collected at a contaminated Detroit River site (Windsor) and at an uncontaminated central Ontario site (Gull River). Samples were split and stored at different temperatures (-20 and -70°C) for 4 mo. Portions of a large (280 g) sample (Detroit River, Windsor) frozen at -20°C were analyzed at 60 day intervals to determine the effect of length of storage time on analytical results. Concentrations were compared by one-way analysis of variance (ANOVA) (Sokal and Rolf, 1969).

2.4 Minimum Useful Sample Size

Minimum reliable sample weight was determined using samples of *Hexagenia* from the Detroit River (Windsor, collected on 23 June 1987), and *Hydropsyche* (Trichoptera: Hydropsychidae) from the Gull River (central Ontario, collected on 18 June 1987,). Triplicates of 5 different subsample weights of insects (0.09, 0.18, 0.38, 0.75 and 1.5 g, dry weight) were analyzed from single collections, and coefficients of variation were calculated for the 30 compounds at each sample weight. Median coefficients of variation were plotted against sample dry weight. The weight at which the change in median approached zero was considered to be the minimum allowable sample weight yielding acceptable variation among triplicates. Percent nondetectable compounds was also plotted against sample dry weight.

2.5 Intersite Comparisons

We compared contaminant concentrations in samples of insects collected at purportedly uncontaminated central Ontario sites to those from the Detroit and St. Clair rivers. We expected to find significantly higher concentrations of all contaminants in samples from Detroit and St. Clair River sites. In addition, similar taxa (*Hexagenia* and Hydropsychidae) were analyzed from different sites along the Detroit and St. Clair rivers to assess possible spatial trends in contaminant distribution along the rivers. To simplify comparisons of organochlorine contaminant levels among samples from various sites, we selected 4 representative compounds (Table 2). Three of the compounds chosen each corresponded to one of 3 major groups of contaminants distinguished by Ciborowski and Corkum's (1988) principal component analyses of data generated by collection and GC analysis of adult aquatic insects at sites along the Detroit and St. Clair rivers. In addition, the pesticide dieldrin was selected to represent pesticide compounds. Site comparisons were performed using one-way ANOVA.

3.0 RESULTS

3.1 Sensitivity

Detailed results of contaminant analyses (site comparisons only) are listed in Tables 3 and 4. Our analyses detected a minimum of 25 of the 30 contaminants studied in all samples from uncontaminated sites. Recovery efficiencies were >90% in spiked samples.

Table 2. Representative compounds used to simplify presentation of the data set and the major groups of compounds they represent.

Representative compound	Group of compounds
PCB 180* (2,2',3,4,4',5,5'-Heptachlorobiphenyl)	Highly chlorinated PCBs (hexa-, hepta-, and octa-chlorobiphenyls)
PCB 66 (2,3',4,4'-Tetrachlorobiphenyl)	Less highly chlorinated PCBs (tetra- and pentachloro-biphenyls)
HCB (Hexachlorobenzene)	Pentachlorobenzene, Hexachlorobenzene, Octachlorostyrene
Dieldrin	Pesticides (Aldrin, Dieldrin, Heptachlor, Heptachlor epoxide, pp'-DDT, pp'-DDE, α-BHC, γ-BHC

* PCB numbering follows Ballschmiter and Zell 1980.

3.2 Sample Storage

Neither storage temperature nor length of storage time significantly influenced analytical results ($p>0.05$). No degradation was noted in animals in either of the fractions of samples split and stored at -20 and -70°C for 4 months, and results of GC analysis were identical. Similarly, a difference of 60 days in length of storage time had no effect on contaminant analyses.

3.3 Minimum Useful Sample Size

Coefficients of variation calculated during minimum sample size experiments and percent non-detectable compounds were plotted for Detroit River *Hexagenia* and Gull River (central Ontario) *Hydropsyche* (Figure 1). Based on these plots, sample dry weights of at least 0.38 g (*Hexagenia*, 25 animals) and 0.75 g (*Hydropsyche*, 170 animals) are sufficient to provide acceptable repeatability of analytical results in samples from highly contaminated and relatively uncontaminated areas, respectively.

3.4 Intersite Comparisons

Concentrations of the representative contaminants in *Hexagenia* collected at 3 sites along the Detroit and St. Clair rivers were compared to those at Balsam Lake (Figure 2). Significantly higher levels of contaminants were detected in samples from the Detroit and St. Clair rivers ($p<0.05$, one way ANOVA). Exceptions were dieldrin at Station 3 (Sombra, St. Clair River) and PCB 66 at Station 4 (Sarnia, St. Clair River), concentrations of which were not significantly different from those at Balsam Lake. In both cases large variation among replicates reduced power of statistical tests. Significant spatial variation in contaminant concentrations in conspecific animals within rivers was

Table 3. Mean (±1 S.E., n=3) concentration of PCBs (µg kg^{-1} dry weight) in aquatic insect adults from various study sites. Systematic names of the compounds are listed in Appendix 1 (ND = non detectable)

TAXON	LOCATION	PCB 6	PCB 18	PCB 19	PCB 31	PCB 52	PCB 44	PCB 88	PCB 87	PCB 97	PCB 101	PCB 110	PCB 118	PCB 138	PCB 141	PCB 153	PCB 170	PCB 180	PCB 182	PCB 194
Trichoptera	Ausable River	ND --	0.14 (0.140)	ND	0.05 (0.050)	1.27 (0.465)	0.60 (1.999)	0.56 (0.096)	0.68 (0.126)	0.42 (0.109)	1.62 (0.455)	0.67 (0.210)	1.75 (0.397)	2.04 (0.688)	0.85 (0.162)	2.81 (0.816)	0.55 (0.279)	1.20 (0.261)	2.09 (0.097)	0.55 (0.266)
Caenis	Lake Scugog	ND --	ND --	ND	1.13 (0.587)	4.20 (0.208)	25.54 (2.270)	2.26 (0.606)	2.04 (0.500)	0.82 (0.215)	10.85 (2.175)	2.72 (0.629)	5.10 (0.861)	22.09 (4.007)	7.77 (1.431)	32.67 (6.597)	7.90 (1.513)	22.90 (3.593)	10.11 (3.096)	3.75 (0.794)
Hexagenia	Balsam Lake	ND	1.57 (0.106)	ND	ND --	0.88 (0.191)	6.53 (2.661)	1.58 (0.461)	1.10 (0.191)	0.64 (0.165)	2.21 (0.218)	1.41 (0.660)	2.01 (0.263)	2.17 (0.530)	0.58 (0.139)	5.58 (1.118)	0.55 (0.038)	1.68 (0.208)	1.16 (0.333)	1.26 (0.198)
Hydropsyche	Gull River	ND	ND --	ND --	8.25 (0.298)	1.66 (0.284)	8.77 (5.096)	0.83 (0.126)	1.00 (0.174)	0.57 (0.065)	2.67 (0.509)	1.00 (0.554)	3.06 (0.114)	5.40 (0.569)	0.76 (0.035)	5.06 (0.484)	1.08 (0.286)	2.35 (0.028)	1.82 (0.249)	0.89 (0.186)
Hexagenia	Sarnia (Stn. 4)	ND --	3.17 (0.376)	ND --	1.68 (0.063)	6.38 (0.995)	13.9 (2.764)	3.25 (0.120)	3.55 (0.516)	2.14 (0.395)	8.69 (0.670)	3.64 (0.280)	7.91 (0.857)	7.70 (0.601)	2.03 (0.366)	13.21 (1.562)	1.46 (0.737)	4.84 (0.605)	1.86 (0.356)	2.91 (0.125)
Hexagenia	Sombra (Stn. 3)	ND	2.08 (0.488)	ND	1.68 (0.106)	16.85 (1.925)	17.79 (1.236)	8.88 (0.107)	5.05 (0.477)	3.67 (0.244)	10.63 (1.212)	5.61 (0.151)	13.57 (0.611)	14.54 (1.165)	6.09 (0.252)	23.19 (1.988)	3.90 (0.130)	9.58 (1.870)	3.33 (0.565)	4.68 (0.542)
Hexagenia	Windsor (Stn. 2)	ND --	7.56 (0.261)	ND	2.75 (0.622)	7.80 (0.145)	17.58 (1.467)	4.61 (0.416)	6.54 (0.571)	5.85 (0.507)	12.92 (0.977)	5.95 (0.655)	10.10 (0.600)	10.14 (0.864)	6.10 (0.180)	21.8 (0.143)	4.80 (0.667)	10.42 (0.526)	7.33 (2.616)	4.69 (0.600)
Trichoptera	R. Canard (Stn. 1)	0.11 (0.101)	7.90 (0.767)	0.18 (0.146)	10.55 (0.586)	20.89 (2.228)	49.21 (8.209)	13.66 (1.293)	62.55 (20.120)	8.60 (0.731)	49.08 (3.379)	16.60 (0.630)	15.54 (7.930)	66.22 (9.866)	23.27 (2.400)	97.55 (11.027)	29.80 (3.726)	63.80 (6.615)	48.52 (6.181)	7.42 (7.412)

Table 4. Mean (±1 S.E., n=3) concentration of pesticides and other organochlorine compounds (µg kg^{-1}dry weight) in representative adult aquatic insect samples at varous sites. Systematic names of the compounds are listed in Appendix 1. (Hept.= Heptachlor, H. Epox.= Heptachlor Epoxide, ND = non-detectable)

TAXON	LOCATION	LIPID (%)	QCB	HCB	OCS	HEPT.	pp'-DDE	pp'-DDT	-BHC	-BHC	ALDRIN	H. EPOX.	DIELDRIN
Trichoptera	Ausable River	10.65 (1.943)	0.05 (0.046)	1.10 (0.093)	0.73 (0.598)	0.10 (0.100)	45.70 (31.255)	3.16 (2.029)	1.49 (0.412)	2.27 (1.339)	0.08 (0.083)	4.65 (1.119)	16.75 (4.447)
Caenis	Lake Scugog	6.86 (0.078)	0.38 (0.197)	0.81 (0.061)	0.12 (0.064)	0.98 (0.984)	28.10 (3.473)	ND --	2.38 (0.882)	0.50 (0.351)	ND --	ND --	3.54 (0.751)
Hexagenia	Balsam Lake	12.56 (1.125)	0.85 (0.183)	1.52 (0.744)	0.33 (0.330)	0.26 (0.263)	21.46 (5.371)	1.03 (1.035)	4.72 (1.083)	3.71 (2.062)	0.31 (0.313)	2.75 (0.160)	3.92 (1.114)
Hydropsyche	Gull River	15.14 (1.005)	0.16 (0.158)	1.88 (0.038)	0.23 (0.106)	0.21 (0.109)	26.31 (0.528)	11.57 (0.808)	8.28 (0.424)	3.53 (0.919)	ND --	7.77 (0.062)	22.40 (1.252)
Hexagenia	Sarnia (Stn. 4)	14.55 (0.536)	3.81 (1.604)	27.48 (3.846)	4.78 (2.323)	ND --	30.40 (5.053)	ND --	14.47 (1.039)	5.70 (0.196)	ND --	8.65 (0.277)	19.87 (1.610)
Hexagenia	Sombra (Stn. 3)	16.54 (0.881)	4.75 (0.503)	69.97 (3.271)	23.13 (3.318)	ND --	36.33 (2.838)	ND --	12.43 (1.881)	6.30 (0.649)	ND --	8.73 (0.749)	12.24 (4.235)
Hexagenia	Windsor (Stn. 2)	21.33 (1.136)	10.23 (0.777)	120.18 (13.882)	16.64 (1.619)	ND --	32.76 (4.399)	ND --	16.71 (1.376)	10.26 (2.447)	ND --	14.94 (0.574)	31.42 (3.294)
Trichoptera	R. Canard (Stn. 1)	22.00 (0.523)	2.39 (0.088)	21.02 (0.934)	30.81 (3.498)	0.63 (0.274)	62.53 (3.339)	27.44 (4.588)	6.49 (0.200)	12.70 (2.209)	ND --	20.95 (1.587)	70.54 (4.224)

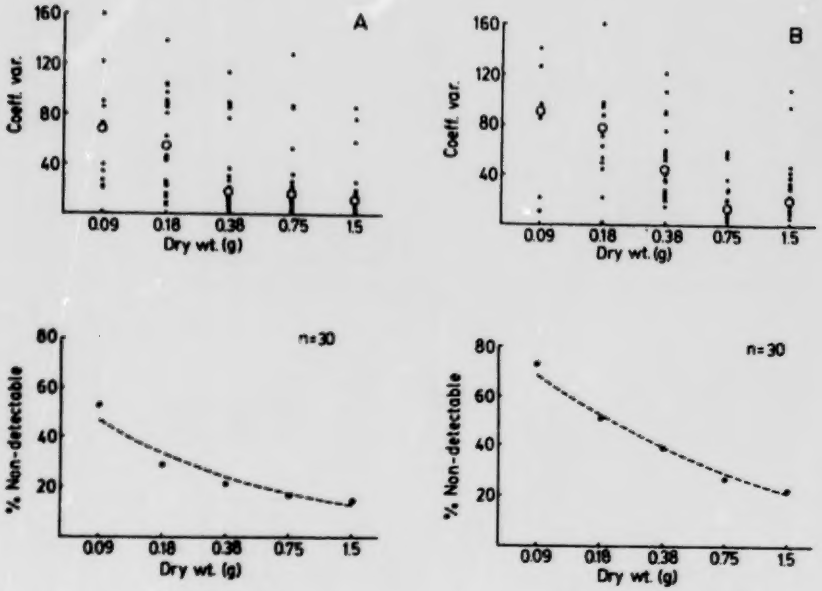

Figure 1. Coefficient of variation (above) and percent nondetectable compounds (below) plotted against sample dry weight for (A) *Hexagenia* at the Detroit River (Windsor), (regression equation: %Nondetectable=25.33 x (Dry weight)EXP(-0.430), and (B) Hydropsychidae at Horseshoe Dam (central Ontario), (regression equation: %Nondetectable=14.23 x (Dry weight)EXP(-0.488). Median values are represented by large open circles.

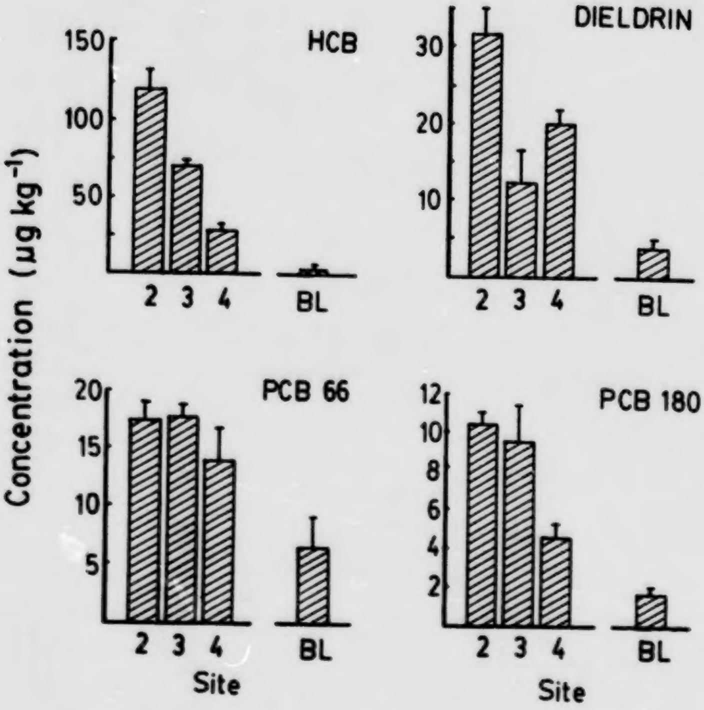

Figure 2. Concentrations of selected contaminants in *Hexagenia* at three Detroit and St. Clair river stations and at Balsam Lake (BL). Insufficient material was collected at Station 1 (River Canard) for analysis. Vertical bars represent 1 S.E. (n=3).

also observed. HCB and PCB concentrations in *Hexagenia* decreased from Station 2 (Detroit River, upstream site) to Station 4 (St. Clair River, upstream site). This pattern corresponds to that reported for PCBs in the sediments at these sites (Pugsley et al. 1985).

Contaminant concentrations were significantly higher in Hydropsychidae at Station 1 (Detroit River) than at central Ontario sites (Ausable River and Gull River, Figure 3). Pesticide concentrations were elevated in samples from the latter sites when compared to levels of other contaminants. The higher concentrations most likely reflect local agricultural activity.

Collections along the Niagara River yielded large numbers of Trichoptera at all sites. Sizes of catches from St. Marys River sites were limited by low temperatures and were considerably smaller. All samples were numerically dominated by Trichoptera, with the exception of one sample collected at the mouth of the St. Marys River, which contained large numbers of the mayfly *Caenis* (Ephemeroptera: Caenidae). Whereas St. Marys River Trichoptera samples were quite diverse, Hydropsychidae and Leptoceridae (Trichoptera) dominated Niagara River samples. Contaminant analyses of these samples are in progress.

4.0 DISCUSSION

4.1 Minimum Sample Size

Results of our analyses indicate that larval aquatic insects accumulate organochlorine compounds and retain sufficient amounts as adults to permit precise analysis by GC. Minimum sample size experiments indicate that sample biomass considered to be small for other invertebrate tissues (~1.2 - 2.5 g. fresh wt.) is adequate to provide acceptable precision in analytical results. This can be explained by the relatively low water content and high lipid content of adult aquatic insects.

4.2 Spatial Variation

Comparisons of contaminated with uncontaminated sites yielded results in accordance with expectations. Exceptions were the pesticides, which exhibited higher levels than expected at uncontaminated sites. Although there were still significant differences between Detroit River and central Ontario sites, this demonstrates the ubiquitous occurrence of these compounds. Variation in contaminant concentrations in the insects among locations paralleled those in the sediments.

Use of samples of adults to infer local larval contaminant concentration requires the assumption that animals have emerged in the immediate vicinity of light traps. However, both active and passive dispersal by flight can occur. Preliminary results of dispersal studies suggest that active dispersal by Ephemeroptera and Trichoptera is limited: In July 1987, Lake St. Clair *Hexagenia* dispersed mean (±1 S.E.) distances of 2570±228 m, and hydropsychid caddisflies travelled 970±178 m (Kovats and Ciborowski, in prep). Passive dispersal by wind is also limited, since aquatic insect adults seldom fly under windy conditions. During inclement weather they cling to the substrate or to vegetation (Johnson 1969). The pronounced spatial trends in adult contamination detected by our study also argue for limited dispersal. Mauck and Olson (1977) and Clements and Kawatski (1984) also reported significant spatial variation in contaminant distribution in Mississippi River *Hexagenia* samples collected 10-20 km apart. We have investigated two alternative trapping methods that could be minimize inclusion of adults

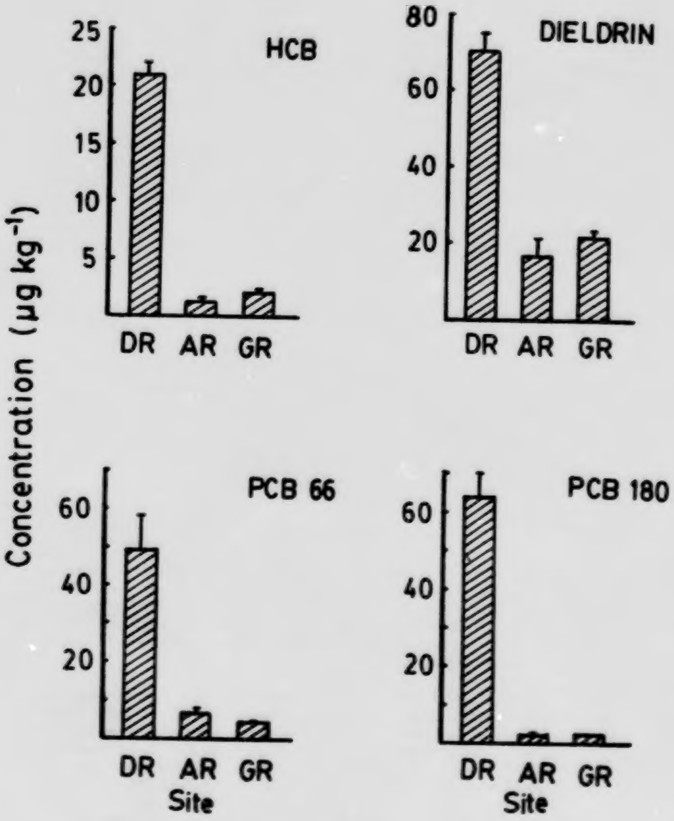

Figure 3. Concentrations of selected contaminants in Trichoptera from the Detroit River and two uncontaminated sites (DR: Detroit River at Windsor, AR: Ausable River, GR: Gull River). Vertical bars represent 1 S.E. (n=3).

5.0 CONCLUSIONS

Overall, adult aquatic insects are sensitive indicators of aquatic contamination and yield reliable data regarding the degree sediment contamination in the area surrounding a given sample station. Though this approach does not provide specific estimates of sediment contaminant concentrations, it is useful in assessing contamination on a larger scale. Animals captured during single collections represent the major aquatic insect taxa of ≤2-5 km stretches of rivers or lakes. Besides its utility in providing monitoring at single sites on a repeating basis, collection and analysis of adult insects may be especially well suited for preliminary surveys of areas previously not studied, due to its relative simplicity and cost-effective nature.

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that have dispersed long distances. Preliminary results of collections using boat-mounted traps, which catch emerging mayfly subimagoes, are promising. We have also employed underwater light traps to collect benthic larvae, but these did not catch appreciable numbers of organisms.

Comparison of our data to similar analyses of Ciborowski and Corkum (1988) reveals the potential for considerable year to year variation in contaminant body burdens in the taxa studied. Though different extraction procedures were used during our analyses, the effect on contaminant concentrations was relatively small (unpublished data). During the period of one year, concentrations of individual PCB congeners increased 2-10 fold in Trichoptera at Station 1 (Detroit River, downstream), while body burdens of other organochlorine compounds remained unchanged. PCB concentrations in *Hexagenia* also increased slightly at Station 2 (Detroit River, upstream), and at Station 3 (St. Clair River, downstream) but dropped by approximately 50% at Station 4 (St. Clair River, upstream). Based on these findings, downstream transport of PCBs is a possibility, though inclusion in upper St. Clair River samples of *Hexagenia* that may have emerged from less contaminated southern Lake Huron areas may account for the relatively low contaminant burdens found in animals at Station 4. Moreover, since both our present study and that of Ciborowski and Corkum (1988) represent single collections from each site, it is possible that the observed temporal variation represents time-specific sampling error. Nevertheless, we believe that the changes in insect contamination reflect temporal trends in sediment contamination. Verification of seasonal trends requires reliable sediment data, precise localization of sample sources, and repeated sampling to estimate the degree of random variation. Analysis of data collected during 1988 will provide more conclusive evaluation of seasonal variability.

4.3 Choice of Taxa and Seasonality

The animals used in our study were chosen primarily on the basis of availability. However, microhabitat differences within the Trichoptera also warrant consideration when selecting taxa for monitoring contaminants since the extent of contaminant uptake and the type of compounds accumulated are dependent upon larval feeding behaviour and substrate preference. Bush et al. (1985) analyzed caddisfly larvae of various species from the Hudson River and found significant differences in PCB body burdens among members of different families. We therefore recommend that samples used for monitoring be ideally composed of single species or at least members of the same family. The Hydropsychidae, composed primarily of riverine species, appear to be the most appropriate group for monitoring contamination in large rivers, and the mayfly *Hexagenia* seems well suited for monitoring lakes.

Though adult aquatic insects emerge throughout the summer months, time of sampling for the taxa selected for contaminant analyses should be carefully chosen. Some trichopteran species are bivoltine, the second generation emerging in late summer (Mackay 1978), having developed from eggs laid in early summer. Sampling for these animals early in the emergence season ensures that contaminants were accumulated during a standard length of time (approx. 1 year), defined by the animal's life cycle. Since *Hexagenia* has a relatively short emergence period, typically 2-3 weeks, sampling for these animals must be precisely timed.

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B10

An Ecosystem Approach to the Monitoring of PCBs in Pristine Ontario Lakes

C.D. Metcalfe and C.R. Macdonald
Environmental and Resource Studies Program
Trent University, Peterborough, Ontario

EXTENDED ABSTRACT

It has been estimated that 7.5 µg to 24 µg of PCBs are deposited per square meter per year from the atmosphere into lakes in the Great Lakes region (Swackhamer and Armstrong 1986, Murphy and Schinsky 1983), and that atmospheric deposition may account for up to 80% of the PCBs entering the Great Lakes (Thomann and Di Toro 1983). There is potential, therefore, for PCB contamination from atmospheric sources in more northern Ontario lakes. Because of their hydrophobic nature, PCBs tend to accumulate within lake biota and lake sediments, and because of their low rates of degradation, these compounds may remain for long periods of time in these environmental compartments. Concentrations of PCBs in some sport fish species have risen to moderate levels (0.5 to 1 ppm) in even "pristine" lakes of Ontario. It is the objective of this study to survey the levels of PCBs in all environmental compartments in seven Ontario lakes to assess the extent of atmospheric contamination, and to indicate the pathways for movement and loss of these compounds in each lake ecosystem.

Lakes chosen for study (Figure 1) are within the Ontario townships of Peterborough (Rice Lake), Durham (Lake Scugog), Stanhope (Boshkung Lake, St. Nora Lake), Oakley (Wood Lake), and Sebastopol (Lake Clear), and from Algonquin Park (Lake Opeongo). The selection of these lakes was based upon trophic status, degree of lake development, and level of PCB contamination in lake biota. Based upon Ontario Ministry of Environment monitoring data for sport fish, PCB contamination in these lakes ranges from low (<10 ppb in smallmouth bass), to medium (10-100 ppb), to high (>100 ppb) levels. Rice Lake and Lake Clear, which have the highest levels of contamination, have known point sources of PCBs, while the other lakes in this study have no distinct sources of these compounds. Three of the study lakes are considered moderately eutrophic (Rice Lake, Lake Scugog, Lake Clear), while the other lakes are oligotrophic. Among the oligotrophic lakes, there is a gradient in terms of lake development, from Boshkung Lake (most developed) to Lake Opeongo (least developed).

Samples of water, suspended particulates, sediment, zooplankton, crayfish, clams, pooled insects, and 5 species of fish (golden shiners, bluntnose minnows, YOY yellow perch, adult yellow perch, smallmouth bass, lake trout) were collected in

1986, 1987, and 1988 for analysis of PCBs. Sediment cores were collected from the deepest deposition zone of each lake using a KB corer, and divided into three sections of 0-3, 3-6, and 6-9 cm. Sediment extracts were prepared by sonication, and sulfur compounds were removed by precipitation with mercury. Lake water samples (54 L) were filtered through 0.3 µm glass fibre filters. The filtrate and filters were extracted with methylene chloride for analysis of PCBs in water and suspended particulates, respectively. Zooplankton were collected using a 276 µ mesh conical net, and other biota were collected using dip nets, minnow traps, seine nets, trap nets, and by angling. Among fish species collected, an attempt was made to sample constant body sizes between lakes, and the ages of fish were estimated from scale readings or published age-length data. Samples of muscle from the mid-dorsal region of each fish and from the tail of crayfish were used for analysis. Biota samples were extracted into hexane using soxhlet apparatus, and lipids were removed by gel permeation chromatography.

Sample extracts were cleaned up and subfractionated by silica gel column chromatography into a "PCB fraction" containing PCBs, DDE, aldrin, heptaclor, and lindane, and two other fractions containing organochlorine pesticides. The PCB fractions were analyzed by high resolution gas chromatography using a Varian 3500 gas chromatograph with a 30 m DB-5 capillary column and EC detector. Rather than analyze all possible PCB congeners, 19 congeners were selected for analysis, ranging from trichlorobiphenyls to decachlorobiphenyl (Table 1). These compounds include most of the major congeners present in commercial PCB mixtures and in environmental samples (Oliver and Niimi 1988, Duinker et al 1988, Norstrom et al. in press).

Analysis of samples from the study lakes supports the original monitoring data used for lake selection. The concentrations of total PCBs (sum of the 19 congeners) in the muscle of adult yellow perch ranged from 8 $ng.g^{-1}$ in Lake Opeongo to 91 $ng.g^{-1}$ in Lake Clear. The same gradient in concentration is observed for all other species of biota in these lakes. In addition to the differences in the concentration of total PCBs in biota from the seven study lakes, the patterns of PCB congeners in the biota differ between lakes (Figure 2). This may reflect different sources of PCB contamination and/or variations in the ecology of the biota in each lake.

The levels of total PCBs in the biota of "pristine" lakes is approximately 4-190 $ng.g^{-1}$. Within lakes there are variations in the pattern of PCB congeners in the biota samples (Figure 3). Principle components analysis indicates that biota in the lower trophic levels have congener patterns most closely resembling Aroclor 1248, while the congener patterns in upper trophic levels most closely resemble Aroclor 1260. There are a broad range of congeners present in the sediments from these lakes (Figure 3).

The data for concentration of PCBs in the various trophic levels of pristine lakes, when expressed on a wet weight basis, indicate that PCB concentration increases with trophic level (Figure 4). These data would seem to support the concept of "biomagnification" of PCBs through the food chain. However, when PCB concentrations are expressed on a lipid weight basis, there is no clear association between trophic level and PCB concentration. The accumulation of PCBs in lake biota may be governed by a variety of factors, including lipid content, feeding ecology, the age of the organism, and the organism's relative metabolic rate.

Detailed analysis of PCB data has been completed for Lake Clear (Macdonald et al, submitted). This lake was accidently contaminated with PCBs in the mid-1970's and contains the highest concentrations of PCBs among the lakes in this study. Concentrations of total PCBs in sediments were highest (597 $ng.g^{-1}$) in the surface section of cores, which indicates that PCB transport in the lake is still a dynamic process. PCBs in lake water and suspended sediments were easily detectable at approximately 1.9 $\mu g.L^{-1}$ and 870 $ng.g^{-1}$ dry weight (1.0 $\mu g.L^{-1}$), respectively. Concentrations of PCBs in biota were high (up to 2900 $ng.g^{-1}$ in lake trout muscle). Unlike the pristine lakes in this study, PCBs in Lake Clear biota were uniform in concentration and congener pattern across all trophic levels. Relationships between bioconcentration factors (BCFs) and octanol-water partition coefficients (log K_{ow}) of individual congeners indicate that very hydrophobic congeners (log K_{ow} >7) are accumulated in biota to a lesser extent than would be predicted from their lipophilicity (Figure 5). Several hypotheses might explain this finding, including reduced bioavailability of very hydrophobic congeners due to binding with dissolved organic or colloidal material in lake water.

The final objectives of this study are to describe the distribution of PCBs and other organochlorine compounds in the seven study lakes, and to establish criteria by which atmospheric deposition of PCBs into lakes can be recognized from point-source discharges of these compounds. We will also use environmental fate models developed by the USEPA (i.e. WASTOX) to simulate atmospheric inputs and the movement of PCBs throughout the ecosystems of these lakes. Using these models, we hope to determine the baseline contamination levels to be expected among biota in pristine Ontario lakes, and to predict the rates of decline (if any) in levels of these compounds among all compartments of the lake ecosystems.

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FIGURE LEGENDS

Figure 1: Location of the seven study lakes in central and eastern Ontario, Canada.

Figure 2: Relative proportions of the PCB congeners analyzed in adult yellow perch from 3 "pristine" study lakes (Wood, Boshkung, Scugog) and from 2 lakes receiving PCBs from point sources (Clear, Rice).

Figure 3: Relative proportions of the PCB congeners analyzed in the sediments and biota from different trophic levels in Boshkung Lake.

Figure 4: Concentrations ($\mu g.g^{-1}$ wet weight) of the PCB congeners analyzed in the biota from different trophic levels in Boshkung Lake.

Figure 5: The relationship between the bioconcentration factors (conc. biota/conc. water) in Lake Clear biota and the octanol-water partition coefficients (log K_{ow}) for individual PCB congeners.

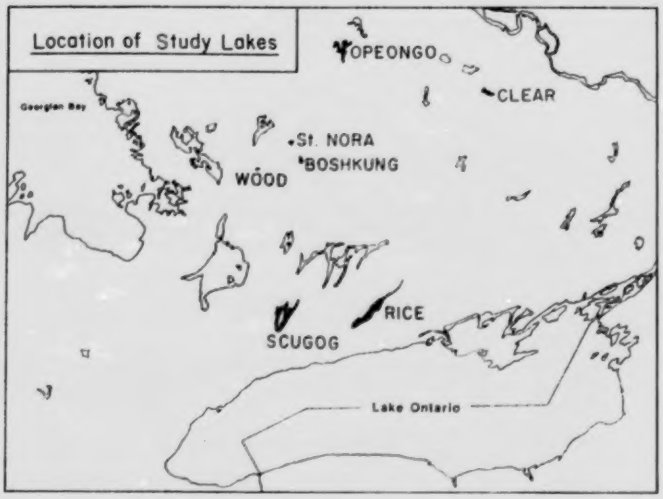
Location of Study Lakes
OPEONGO
CLEAR
Georgian Bay
St. NORA
BOSHKUNG
WOOD
RICE
SCUGOG
Lake Ontario

FIGURE 1

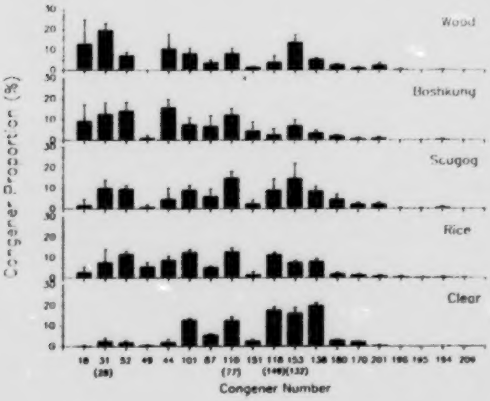

FIGURE 2

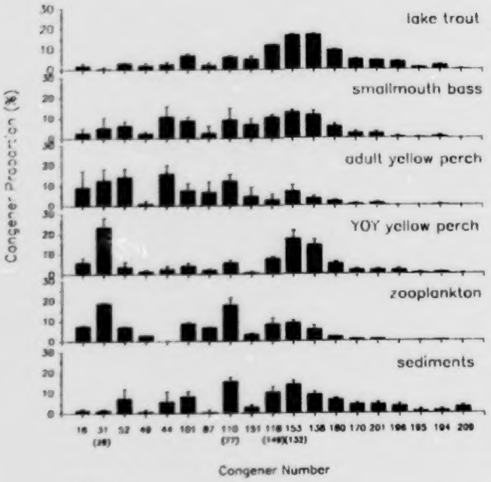

FIGURE 5

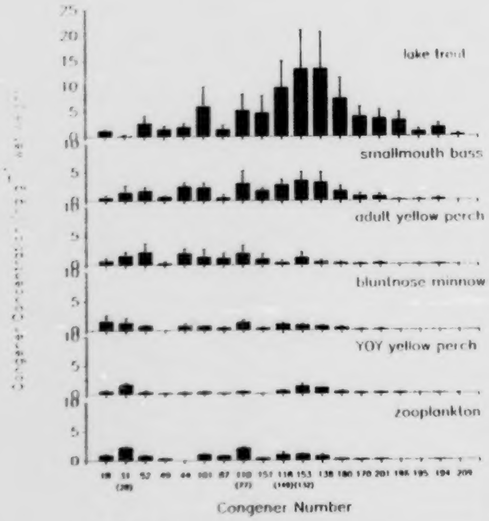

FIGURE 6

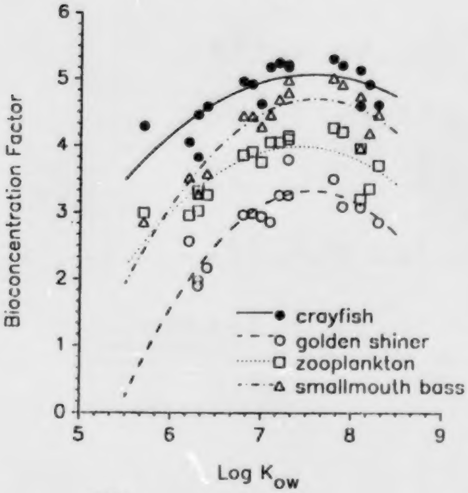

FIGURE 5

Table 1 - Summary of PCB congeners analysed in seven study lakes. Brackets indicate co-eluting congeners.

Congener Number[1]	Chlorine Number	log K_{ow}[2]	Chlorine Position
18	3	5.24	2,2',5
31 (28)	3(3)	5.67(5.67)	2,4',5 (2,4,4')
52	4	5.84	2,2',5,5'
49	4	5.85	2,2',4,5'
44	4	5.75	2,2',3,5'
101	5	6.38	2,2',4,5,5'
87	5	6.29	2,2',3,4,5'
110(77)	5(4)	6.48(6.36)	2,3,3',4',6 (3,4,3',4')
151	6	6.64	2,2',3,5,5',6
118(149)	5(6)	6.74 (6.67)	2,3'4,4',5 (2,2',3,4',5',6)
153(132)	6(6)	6.92 (6.58)	2,2',4,4',5,5' (2,3,4,2',3',6')
138	6	6.83	2,2',3,4,4',5'
180	7	7.36	2,2',3,4,4',5,5'
1.0	7	7.27	2,2',3,3',4,4',5
201	8	7.62	2,2',3,3',4,5,5',6'
196	8	7.65	2,2',3,3',4,4',5,6'
195	8	7.56	2,2',3,3',4,4',5,6
194	8	7.80	2,2',3,3',4,4',5,5'
209	10	8.18	2,2',3,3',4,4',5,5',6,6'

[1] - IUPAC numbering system for PCB congeners.
[2] - Log K_{ow} values from Hawker and Connell (1988).

B11

METAL CONTAMINATION OF WETLAND FOODCHAINS IN THE BAY OF QUINTE, ONTARIO

A.A. Crowder

W. Dushenko

J. Greig

Queen's University

Department of Biology

Kingston, Ontario K7L 3N6

ABSTRACT

Diversity and biomass of aquatic macrophytes diminished during hypereutrophication of the Bay of Quinte, Lake Ontario, during the 1960s and have not recovered. Numbers of waterfowl and mammals in wetlands in the Bay are also low. It was hypothesized that the wetlands are contaminated by metals derived from mines in the Moira Valley, compounding the stress of eutrophication. Possible contaminants analysed in samples of sediment have included Ag, As, Al, Cd, Co, Cl, Cu, Hg, Mg, Mn, Na, Ni, Pb, Ti, V and Zn. Nutrient concentrations in sediment were also measured (N, P, K, Ca and Mg). Elemental concentrations in emergent and submerged plants and in snails have been analysed, using neutron activation analysis and atomic absorption spectrophotometry, to test for transfer of contaminants up food chains. Cover classes of submerged plants at sites around the Bay were correlated with metals and nutrient concentrations, and also with limiting factors such as exposure and slope of shores, organic carbonates and silt content of sediments, and pH.

Significant differences occur between wetlands in the Moira River area and in Hay Bay, about 20 km east. Concentration of As, Co, Na, Mn and Pb are higher in Hay Bay. concentrations of As (2.1-4.4 ppm), Cr (29.2-46.6 ppm), and Cu (10.3-25.1 ppm) are potentially toxic, while sediment Hg is not (< 0.2 ppm). Submerged plants in the Moira area contain significantly higher concentrations of As and Mn (means 4.6-6.8 ppm As; 656-703 ppm Mn). One sampled snail species (*Stagnicola elodes*) accumulates 34.93^{+} - 12.44 ppm Cu. Concentrations of Mn and Al in snails are also elevated, but show high variance within species.

INTRODUCTION

The International Joint Commission has recognized two areas on the north shore of Lake Ontario which need remedial action programmes, Hamilton Harbour and the Bay of Quinte. The latter has historically been the most productive fishery in Lake Ontario. The shore of the Bay has extensive wetlands, which were estimated in 1979 as 2824 ha of emergent marshes and 627 ha of submerged and floating-leaved vegetation (Crowder and Bristow 1986). These wetlands affect the aquatic ecosystem, by providing food and/or habitat for invertebrates, amphibians, fish, birds and mammals. The wetlands are also used for muskrat trapping and for non-consumptive activity such as bird-watching. (Cf. National Wetlands Working Group 1988, Ch. 6).

During the early part of this century eutrophication of the Bay of Quinte increased, culminating in a hypereutrophic state in the 1960's, which led to control of point sources of phosphorus in the 1970's (Minns et al. 1986). The weedbeds of submerged macrophytes at first increased and then sharply declined in biomass during the hypereutrophic phase. Such declines, combined with lowered diversity and invasion by non-native plants, have been characteristic of eutrophic sites both in north America and in Europe (Crowder and Bristow 1988). By the 1970's the area of weedbeds had become limited by light penetration (because of dense algae), and diversity and biomass were low in comparison with regional norms. In the emergent cattail stands diversity was also low, and both weedbeds and marshes were visited by fewer migratory birds than are expected in such habitats in eastern Ontario (Crowder et al. 1986).

Metal contamination frequently accompanies eutrophication, as a result of industry, urbanization and agricultural run-off; Hamilton Harbour is an obvious case of eutrophication with simultaneous metal contamination of sediments. In the Bay of Quinte sediment in deep water is known to contain elevated concentrations of Cd, Hg, Pb and Zn (Sly 1986). An obvious source of metals is the inactive mines of the Moira Valley, where contaminated sediments containing Pb, Co, Ni, Cu, As, Ag and Hg were analysed by Mudroch and Capobianco (1979, 1980) and by Pealke et al. (1982).

In the area near Belleville and Big Bay, submerged weedbeds during the decade 1972-82 had less biomass and cover than in other areas of the Bay of Quinte (Crowder and Bristow 1986). We therefore hypothesized that this area may have received metals from the Moira valley over a long period of time. This project, supported by the Ontario Ministry of the Environment and the World Wildlife Fund, is designed to test this hypothesis, and to estimate the likelihood of transfer of metals in local foodchains. The project will also test for organic pollutants in wetlands, during 1988-89.

Three sets of data have been collected. The first comes from Bend Bay, a riverine wetland below the mining area on the Moira River, where sediment, plants and animals could be collected to represent a ´worst possible case´.

The second consisted of 49 sites along the shoreline, at places with and without vegetation. These sites were on the north and south shores of the Bay, which is about 90 km long. This set was designed to give an overview of near-shore conditions throughout the Bay and is referred to as near-shore samples.

The third set was collected within four wetlands with submergent and emergent vegetation. Two wetlands, at Point Anne, were as close as possible to the mouth of the Moira River, and were chosen to represent sites with potential contamination over a long period of time. The second pair of wetlands, matching the first in aspect and vegetation structure, were in Hay Bay, about 20 km downstream from the Moira River, and therefore presumably free from contamination. This set contained both near-shore and on-shore samples. Sites have been mapped in Crowder et al. 1988a & b. Results including analyses of sediments, plants and animals have been published in Crowder *et al.*, 1988a, b and c. This report therefore condenses information based on field work done in 1987. Some analyses are not yet complete, and will be published with results of the second phase of research (which will include seasonal measurements of uptake, fractionation (speciation) of metals and analysis of organic contaminants. Species reported here include *Myriophyllum spicatum*, Eurasian milfoil, which is the most common submerged plant in the Bay and *Vallisneria americana*, wild celery, a major food for ducks (Crowder and Bristow 1988). Consumer organisms had to be species found abundantly and not likely to migrate far, - those reported here are the snails *Stagnicola elodes* and *Planorbella trivolvis*, the most abundant molluscs found in many sites. Both species of snail are eaten by fish and birds.

Although the Bay of Quinte has been studied intensively (Minns et al. 1986), the hydrology of its shorelines and wetlands are not well known. It is not known whether elements draining from the Moira tailings have been in particulate form or in solution. The marshes are subject to wind set-up and to small seiches, but their intensity and frequency are unknown, as is their potential for sediment transport. It is not known whether water carries sediment up into the marshes during the melt, or erodes them; the complexity of the shoreline presumably could allow both occurences simultaneously, but there may be temporal variance in amount and velocity of the meltwater, and in the ice in the marshes. Geis (1985) has described 'ice-foot' phenomena in the St. Lawrence River which affect sediment and plants, and similar events were observed in Hay Bay during 1988. In on-shore sites spatial distribution of metals has been studied, to try to elucidate possible depositional/erosional patterns, and will be reported later.

METHODS

Sediment collection

At near-shore sites sediment was collected with an Ekman dredge, in a depth of 0.5 m of water. At on-shore sites (e.g. Point Anne, Hay Bay) 10 composite samples of 6 cores each were collected, using plastic coring tubes or shovels. Only the rooting zone, the uppermost 10 cm of sediment, was utilized. Field measurements of pH were made. Particle size analysis, using sieve and hydrometer techniques, was done in the Hydrology Department of Loyalist College at Belleville. Loss of ignition (organic carbon) was measured after 2 h at 420° C. All sediment was air-dried and sieved to 2 mm diameter before element analysis.

Collection of plants and measurement of vegetation cover

At near-shore sites *Myriophyllum spicatum* and *Vallisneria americana* were collected as close as possible to sediment collection points, where vegetation occurred. In the on-shore sites composite samples of 10 plants of each species were collected at each coring point. Plants were washed and placed in a cooler in plastic bags in the field. Roots and shoots were separated for biomass estimation, air-dried at 70° C and ground to a diameter of 1 mm prior to analysis.

Cover was estimated as four classes, ranging from 0 = no plants to 4 = dense cover, in late summer.

Collection of snails.

Thirty-five *Planorbella trivolvis* and 35 *Stagnicola elodes* were collected from each on-shore site. Snails were kept in deionized water for 24 h for digestive clearance, then frozen. Tissue was later removed from shells, and dried at 90° C. Small samples were not ground.

Element analysis

i. Neutron activation analysis (NAA)

Protocol and standardization of NAA using a Slowpoke-2 reactor at Royal Military College, Kingston, were developed by Dr. J. Poland (Queen's University Analytical Services) and Dr. P. Beeley. Two aliquots of a dry sample of sediment, plant or animal tissue were weighed in a 7 mL plastic vial and heat-sealed. Samples were irradiated in an automated handling system. One sample received a 1-3 minute irradiation at a neutron flux of 5 x 10^{11} n cm^{-2} sec^{-1}, followed by a decay time of 1 - 10 m to detect short-lived nuclides (e.g. Al, Ca, Cl, Cu, K, Mg, Mn, Na, Ti and V). The second sample was irradiated for 2 h, and left for a decay period of (a) 100 h to detect As, Br, K, La, Na, Sb,, and Sc and (b) 250 h to detect Ba, Co, Cf, Cs, Fe, Hf, Rb, Ta, Th, U and Zn.

ii Atomic absorption spectrophotometry

Extraction was done by two methods:

(a) 1-2 g of dry material were weighed into a teflon beaker and dissolved using HNO_3, $HClO_4$ and HF, followed by re-acidification using 20% HCl. Standards were prepared in 20% HCl, and Ag, Cd, Co, Cu, Ni, Pb and Zn determined by flame AAS. (b) Digestion with HNO_3 and H_2O_2 was done in teflon 'bombs' for 12 h at 130°. No significant difference was found between extractions (a) and (b), therefore the first method was used alone for later tests. Determination of Hg used a cold vapour technique and As was measured using a hydride generation system.

Certified reference materials for standardizing analyses were BCSS NRCC marine sediment, NBS SRM 1572 citrus leaves and TORT-1 NRCC invertebrate tissue.

Statistical analysis

Statgraphics programmes (references) were used for data handling and analysis. Significance was indicated by p values less than 0.05.

RESULTS AND DISCUSSIONS

Cover of plants in near-shore sites.

Cover was estimated as 4 classes and was positively correlated with silt and with organic matter (loss on ignition) in sediment. It was negatively correlated with sand and fetch; fetch is a measure of the exposure of a site to wind and wave action. High energy shores have sand deposition and tend to be too disturbed for plant growth. Positive correlations with silt and organic matter indicate that at sites where accumulation can occur, both of fine particles and plant debris, dense growth of plants is possible. (Cf. Crowder et al. 1988b).

Cover was also related to pH, possibly because sites accumulating organic debris become slightly more acid. Significant correlations were found between cover classes and Pb and Zn in sediment and the nutrients P and Mg; these correlations were positive but there was a negative relationship with Cu. Correlation coefficients are shown in Table 1.

Elements in near-shore sediments

Ranges and means of selected elements are given in Table 2. Limitations and advantages of technique have been discussed in Crowder *et al.* (1988a). The broad range of elements analysed by NAA allowed an overview of the 49 sites to be made. Some elements which were expected to occur were generally near limits of detectability, notably Hg and Cd. Absence of these potentially toxic elements indicates a major difference between the near-shore sediments and the sites in deep water described by Sly (1986). They also differ strongly from sites up the Moira valley described by Mudroch and Capobianco (1979). The range for Cd, less than 1.0

to 2.9 ppm, is comparable to surface values in depositional zones in Lake Ontario (0.1-6.2 ppm) reported by Mudroch *et al.* (1988). Their value for Hg in surface sediments in depositional basins was 0.140-3.95 ppm, and the mean Quinte wetland values were all less than 0.1 ppm.

Chromium had a mean value of 31 ppm; its range of 14-68 ppm is within the limits (8-133 ppm) reported by Mudroch *et al.* (1988). Copper ranged from 24 to 490 ppm, whereas the surface range in depositional basins given by Mudroch *et al.* (1988) is 26 - 109 ppm. In wetlands Glooschenko (1988) has reported high variance, and in agricultural soil a mean of 25 ppm for Cu. The sites with high copper are therefore potentially toxic.

Nickel had a mean of 20.9 ppm, which is at the lower value reported by Mudroch *et al.* (1988); this value is very close to those in Rattray Marsh and Coote's Paradise reported by Glooschenko (23 -29 ppm) and higher than the mean for agricultural soil (16 ppm). Zinc, like Cu, had very high variance, with a mean of 59.7 ppm. Mudroch *et al.* (1988) also show very large ranges (e.g. 14-1225 ppm in embayments) while the Quinte wetlands are again very similar to the marshes described by Glooschenko (1978), with 29-88 ppm. Lead, with a mean of 16 ppm, is relatively low except at the upper end of the range, 55 ppm. Mudroch *et al.* (1988) give values of 7-285 ppm for depositional basins, and 7-169 ppm in river mouths.

Sodium ranged from 0.05-4.7 percent, a high range for a freshwater system. Chlorine was also high at some sites but did not coincide with the peaks for Na. Salinity controls plant diversity in coastal marshes in the United States, with the lowest species richness in saline sites (Larrick and Chabreck 1978), therefore the Quinte values indicate possible conditions of low diversity for plants. Sodium is high in some tailings in the Moira valley, but both Na and Cl could be derived from road salt and snow piles.

Analysis of As from the near-shore sites is not yet complete.

Several distributional patterns were apparent (Fig. 1). Nickel was high only at Bend Bay, and in low concentration from the mouth of the Moira River downstream. Copper was highest at Bend Bay but also peaked in Hay Bay and Sucker Creek. Zinc was concentrated at several sites, although showing some general attenuation downstream. Lead seems to have an urban origin, being highest at the mouth of the Moira River rather than close to the mines. Copper was not closely related to particle size, but Zn, Ni and Pb were positively correlated with fines, which could explain their distribution patterns. (Table 3). Lead and Zn, and Cu and Ni tended to co-occur.

Elements in on-shore sites

The greatest difference from near-shore sites was the higher organic content (53% mean loss on ignition); the mean value for near-shore sites was 16%.

Arsenic at on-shore sites is significantly greater in concentration at Point Anne marshes than downstream in Hay Bay marshes (Table 4). Arsenic values were strongly and positively correlated with organic matter and weakly correlated with Pb and Zn. The significant difference between these wetlands supports the hypothesis that mine drainage has affected wetlands in the upper Bay of Quinte. In comparison with Bend Bay, however, the As values are a magnitude smaller; values from Bend Bay, Point Anne and Hay Bay marshes are shown in Table 4. Cu and Ni were also higher at Bend Bay than in all the Bay of Quinte marshes. There was more Pb and Ni near Belleville than in Hay Bay, but Cu was similar in both areas.

Metals in plants

Concentrations of As in both *Myriophyllum spicatum* and *Vallisneria americana* were significantly higher in Point Anne than Hay Bay wetlands, reflecting sediment values. (Table 5) The plants had specific differences in uptake, for example Pb was significantly higher in *Myriophyllum* and Na in *Vallisneria* at both areas (Table 6). In both species, and in both wetlands, Hg was less than 0.2 ppm. Copper, which was negatively related to cover in near-shore sites, had mean values in plants between 4.0 and 5.3 ug. g -1. in both areas.

Metal content of snails.

Planorbella trivolvis and *Stagnicola elodes* accumulated significantly different amounts of Ca, Mg, Al, Cu, V, K, and Na in their tissues. *P. trivolvis* had higher Ca and Mg, and *S. elodes* had high Al and Cu; its mean Cu content was 34.9 ug.g^{-1}. Arsenic in snails in the onshore marshes ranged from 1.8-4.1 ug.g^{-1} in *P. trivolvis* and from 2.5-12.9 ug.g^{-1} in *S. elodes*, with the higher value in Hay Bay. *Planorbella trivolvis* can accumulate higher amounts where contamination is greater, as the mean value from Bend Bay was 11 ug.g^{-1} with a range of 3.1-13.2 ug.g^{-1}.

Potential toxicity in food chains.

The detailed comparison of wetlands at Point Anne and Hay Bay showed that As is present in the area of Moira drainage, in elevated amounts in both sediment and submerged plants. In the snails, however, higher As values were found in Hay Bay.

Copper is high in the lower bay, and did not differ in plants at the two onshore areas. The elevated Cu in Hay Bay was also found in snails, but only in *Stagnicola elodes*. The specificity of uptake was shown by the highest As values being found in the other sampled species, *Planorbella trivolvis*.

So far, our research indicates that of the seven metals thought likely to be of concern, As and Cu are most important in the wetlands. They occur in sufficiently elevated concentrations at some sites in the Bay to be taken up at elevated levels by plants and molluscs, and therefore passed on to upper trophic levels. In both plants and molluscs the patterns of uptake are species - specific and metal specific.

While some metals (notably As) have the predicted attenuation pattern away from the Moira, several unexpected distributions have been found, such as Pb at Belleville, Cu in Hay Bay and minor concentrations of several metals at Creek mouths.

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Table 1. Correlation coefficients for vegetation cover and metals.

	Fetch	Silt(%)	Sand(%)	LOI^{a}	pH^{b}
Vegetation Cover	-.461 P=.001	.458 P<.001	-.725 P<0.001	.355 P=.012	-.565 P<.001

	Cu	Ni	Pb	Zn
Vegetation Cover	-.19 NS	.232 NS	.437 P=.002	.320 P=.03

[a] loss on ignition (420°C)
[b] in sediment

Table 2. Mean and range of element concentrations in sediment from near-shore sites (n=49) in the Bay of Quinte. SD= standard deviation.

a) Element

	Al %	Ba ppm	Ca ppm	Cl ppm	Cd[a] ppm	Co ppm	Cr ppm	Cu ppm	Fe %
Mean	3.2	466	9.0	258	<1.0	19.7	31.0	23.3	1.87
SD	1.5	182	17.1	107	-	15.3	13.7	68.4	0.80
Min.	0.1	109	0.39	50	<1.0	2.2	7.4	2.0	0.57
Max.	9.7	951	119.2	673	2.9	68.6	68.6	490.0	4.46

b)

	Hg[a] ppm	La ppm	Mg %	Mn ppm	Na %	Ni ppm	Pb ppm	V ppm	Zn ppm
Mean	<0.1	21.3	0.89	436	1.31	20.9	16.0	35.9	59.7
SD	-	18.4	0.54	235	0.76	10.3	11.4	19.1	45.1
Min	<0.1	3.6	0.03	43	0.05	7.0	5.0	2.3	10.0
Max	<0.1	127.1	3.66	1449	4.72	56.0	55.0	108.2	270.0

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[a] levels below detection limits precluded determinations of standard deviations.

Table 3. Correlation of metals in sediment with physical and chemical factors at 49 near-shore sites in the Bay of Quinte.

	Silt(%)	Sand(%)	Clay(%)	LOI[a] (nearshore)	pH[b]
Cu	NS	.25 P=.08	NS	NS	.31 P=.04
Ni	.31 P=.03	NS	.25 P=.08	NS	NS
Pb	.43 P=.003	NS	.31 P=.03	NS	NS
Zn	.45 P=.001	NS	NS	NS	NS

[a] loss on ignition (420°C)
[b] in sediment

Table 4. Comparison of mean values of metals in sediment at Bend Bay, Belleville and Hay Bay marshes (on-shore sites). Values in brackets are standard deviations.

Location	As (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)	Zn (ppm)
Bend Bay (n=2)	180.3 (±41.2)	60.5 (±16.5)	334 (±249)	36.5 (±7.8)	101 (±26.9)
Belleville (n=10)	9.4 (±2.0)	31.5 (±8.6)	16.5 (±5.2)	31.9 (±12.5)	126 (±60.3)
Hay Bay (n=14)	3.0 (±1.0)	32.4 (±12.4)	26.1 (±8.1)	18.5 (±8.0)	89.1 (±25.4)

Table 5. Comparison of metals in *Myriophyllum spicatum* and *Vallisneria americana* in on-shore sites (Belleville/Pt. Anne and Hay Bay).

	Mean Tissue Levels							
Region/Species	As ug/g	Cr ug/g	Cu ug/g	Hg ug/g	Mn ug/g	Na %	Ni ug/g	Pb ug/g
Belleville (n=4)								
M. spicatum	4.6	33	4.0	<0.2	703	0.66	11.9	6.0
V. americana	6.8	28	5.3	<0.2	656	2.43	13.3	<1.0
Hay Bay (n=4)								
M. spicatum	1.2	23	4.2	<0.2	420	0.60	10.3	6.5
V. americana	1.9	29	5.0	<0.2	522	2.32	9.5	<1.0

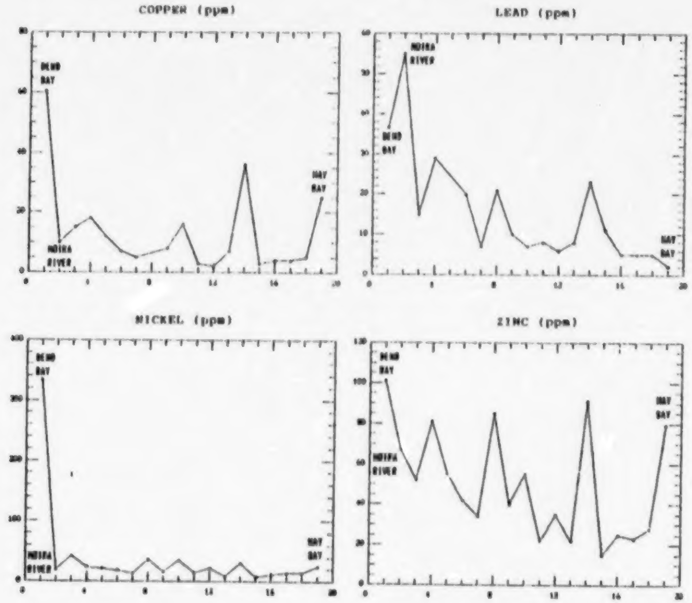

Fig. 1. Distributional patterns of Cu, Pb, Ni and Zn in sediments from Bend Bay and north shore sites from the Bay of Quinte. Numbers on the horizontal axis order the sites by distance from Bend Bay: actual distance from Bend Bay to site 20 is ± 90 km.

B12

AN OVERVIEW OF AQUATIC ENVIRONMENTAL RESEARCH IN QUEBEC;
M. Slivitsky, INRS-EAU, Ste. Foy, Quebec.

MANUSCRIPT NOT AVAILABLE

B13

DEVELOPMENT OF AN IMPROVED SYSTEM FOR THE APPLICATION OF POWDERED ACTIVATED CARBON IN WATER TREATMENT PLANTS

H. Donison, A. Benedek, and J.J. Bancsi, Zenon Environmental Inc.
and S. Beszedits

In the past decade the potential for contamination of Ontario drinking water sources by toxic organic pollutants has become increasingly apparent. Many sources have chronic low level contamination. Other sources could potentially be contaminated with high levels for a short time period as a result of an accidental spill. As a result many communities in Ontario are either already using or are proposing to use powdered activated carbon (PAC). ZENON Environmental Inc. has undertaken a study for the Ontario Ministry of the Environment to develop process design criteria for carbon contacting and to undertake the basic research necessary to ensure that the addition of PAC, if deemed desirable, proceeds in an optimum manner for all concerned.

At the time of writing approximately ten months of a 24 month schedule has been completed. Initial study included a detailed literature review of the application of PAC for organic removal in water treatment and a search of the wastewater or process water treatment literature for PAC reactor designs with potential application for drinking water treatment. Process design criteria for application of PAC in drinking water treatment for the removal of toxic organic contaminants were developed and two PAC contacting systems were selected from potential contacting systems. These two contactor systems are being developed and evaluated in bench scale systems and the best system will be demonstrated at pilot scale in 1989. This paper summarizes the results of the literature review and discusses the process design criteria and the selection of PAC contactor systems for evaluation.

CONVENTIONAL PAC USAGE IN WATER TREATMENT

The application of powdered activated carbon (PAC) is a routine practice in many water treatment plants around the world. Approximately 90% of water treatment plants worldwide that use activated carbon do so in the powdered form (Faust and Aly, 1983). PAC is used by most of the EEC countries primarily as an emergency measure in case of temporary pollution of the raw water source (Water Research Centre, 1977). Large amounts of PAC are also used, particularly in the Netherlands, for taste and odour removal. In the United Kingdom facilities for dosing PAC are available at approximately 100 water treatment works (Hayes and Whitford, 1982). In recent years there has been a growing trend toward the installation of granular activated carbon (GAC) adsorption systems for treatment of low level contamination.

PAC is usually added before coagulation or immediately before the filters. The optimum point of application should allow adequate dispersion of the carbon and sufficient contact time to ensure maximum adsorption. When relatively low doses are required (i.e. 10 mg/L or less) and filter runs are long enough, PAC is added immediately ahead of the filters. Experiences have also demonstrated that a given amount of PAC is more effective when deposited on the filter; however, care must be exercised to prevent PAC from passing the filter in this situation. Moreover, to improve the filter's ability to retain carbon particles, a small dose of polymer is usually added. When relatively high doses of PAC are required, or when filter runs are already short, PAC is added ahead of the coagulation-flocculation zone in order that as much may be removed by sedimentation (if conventional treatment is employed) as possible.

While in normal practice typical PAC doses required are in the range of 3 to 15 mg/L, in some instances, doses as high as 240 mg/L have been utilized. The PAC dose that can be effectively used for direct or in-line filtration is usually limited to between 10 and 15 mg/L. Dosage in most water treatment plants is established by trial and error. When large amounts of PAC are required to reduce the concentration of organics, adding the carbon in two steps (i.e. split dose) usually results in reducing the total quantity of carbon required.

Although PAC may be fed as either a dry chemical or a slurry, it is more preferable to store PAC and feed it as a slurry. Slurry tanks are usually constructed of concrete and equipped with a mixer to maintain carbon in suspension. Volumetric metering or proportioning pumps are best to use in feeding.

ALTERNATIVE PAC CONTACTING AND SEPARATION METHODS

The survey of published literature did not reveal any alternative PAC contacting and separating methods being used or developed for drinking water treatment. However, a wide variety of systems which are being used or developed for wastewater treatment were identified.

PAC treatment of water or wastewater in the simplest sense involves the intimate contact of the carbon with the stream for a sufficiently long period to attain as much removal of undesirable constituents as possible followed by separation of the carbon from the liquid phase to prevent carryover of carbon fines in the discharge and/or to recover the spent carbon for regeneration. These two fundamental steps are illustrated in Figure 1. As indicated in Figure 1, contacting PAC with the stream to be treated can be achieved several ways, e.g. flash mix chamber, fluidized bed, pipe reactor, etc. while carbon separation can be accomplished by sedimentation, flocculation-sedimentation, air flotation, foam fractionation, filtration, centrifugation, etc. Carbon contact with the stream and separation may also be carried out in a single unit such as the reactor-clarifier depicted in Figure 2. Hence, it is evident from these two figures that many combinations of PAC contact and separation operations are possible.

Although carbon contacting and carbon separation processes can be considered individually to identify the most viable system for a particular application, it is more appropriate to consider these two basic operations as part of the overall treatment scheme as depicted in Figure 3.

Based on the literature review, process knowledge and consultation with MOE staff, twelve reactor systems were selected for initial evaluation. These systems are shown in Table 1.

CRITERIA FOR EVALUATION

The reactor alternatives which were identified as a result of the literature survey were evaluated with respect to a set of criteria which were established to ensure optimization of the adsorption process and to meet the requirements of treatment plant operators and the MOE. Table 2 summarizes these criteria for optimum PAC adsorption. The essential requirements are effective contact of the PAC with the water stream to allow adsorption to occur and effective separation of the PAC from the water to remove the contaminants after the adsorption.

When PAC is brought into contact with the water stream dissolved pollutants are removed by adsorption. This is an equilibrium process and thus the capacity of carbon to adsorb a particular compound in the water is dependent on the concentration of that compound in the aqueous phase. The relationship between capacity (q) and equilibrium concentration (C) is given by isotherm data and can be expressed as:

$$q = KC^{1/n}$$

where K and 1/n are experimentally determined for a specific compound over a certain range of concentration.

In a plugflow reactor, the initial layer of carbon adsorbs contaminants until it reaches equilibrium with the influent contaminant concentration. When this equilibrium condition is reached, the removal capacity of the carbon is exhausted. This equilibration process progresses through the carbon bed as each layer reaches equilibrium with the influent concentration. This steady exhaustion of successive carbon layers at the influent concentration results in optimum utilization of the carbon, as the carbon is in equilibrium with the influent concentration, and very low concentrations of contaminants in the produced water, as the effluent is in equilibrium with virtually unused carbon.

The conventional PAC application in drinking water is a completely mixed reactor. In this system the only adsorption equilibrium is between the carbon and the final effluent concentration. Thus the carbon is not used efficiently and the product water has a relatively high concentration of contaminants. In a reactor where the PAC contact is not plugflow if two or more reactors can be used in series and the PAC moved counter currently through the series PAC adsorption capacity and effluent quality can be optimized.

Thus an essential criteria for selection is that contact be stratified (plugflow or multistaged).

In any reactor PAC contact time must be optimized to maximize adsorption. Kinetic studies have in the past focused on granular activated carbon. For granular carbon adsorption optimum contact time is generally greater than five minutes however as the particle size decreases optimum contact time decreases and thus a contact time of one or two minutes may be sufficient for powdered activated carbon. For selection criteria, one minute was used, however, experimental testing is required to further define this.

The third essential criteria is that the PAC be adequately retained by the reactor to prevent deposition of PAC downstream in the water treatment process or distribution system.

Another essential requirement for the PAC system is that it must be able to adsorb occasional high levels of contaminants in response to a chemical spill in the water source. This capacity may be automatic or in response to adjustment of operating conditions by operator.

Other essential criteria are that the system be adaptable to existing facilities, the process must operate reliably and the system must be mechanically reliable.

The "desirable" criteria were included in this analysis as conditions which would have benefits for the MOE or treatment plant operators but were not necessary to an improved PAC contactor system. The first "desirable" criteria was that the system be portable so that it could be moved to a facility encountering temporary contaminant problems. The second desirable criteria is that the PAC be recovered in a form suitable for regeneration.

The third class of criteria were the cost criteria which were included to ensure that any system selected for further investigation have reasonable capital, labour, and operation and maintenance costs associated with it.

REACTOR SYSTEM EVALUATION

The twelve PAC systems were evaluated with respect to the established criteria using information obtained from the literature, information obtained in bench scale testing and best engineering judgement. Four of the systems met the essential criteria. These were 1) Solids Contact Clarifier, 2) Volatilization/Gas Phase Adsorption, 3) Down Flow Filter and 4) Multistage Crossflow Filtration. With respect to the desirable criteria it was uncertain if any of these systems could be made readily portable. All systems except the solids contact clarifier would produce exhausted PAC in a form suitable for regeneration. With respect to costs, the solids contact clarifier is expected to be the least expensive alternative, while the other systems would depend on innovation and optimization to bring the costs to an acceptable level.

For the purposes of this study, the downflow filter and the multistage crossflow filter were chosen for further study. The solids contact clarifier has been well studied for wastewater treatment and is being optimized in on-going studies by V. Snoeyink (Snoeyink 1988). The volatilization/gas phase adsorption system is limited in application to compounds which are efficiently air strippable and thus was eliminated from further study on this basis.

Bench scale testing of PAC downflow filtration and multistage crossflow filtration is currently on-going. A technical and economic evaluation of these two systems will be made based on the results of these test.

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TABLE 1

ALTERNATIVE PAC SYSTEMS

System	Contacting System	Separating System	Combined Contacting Separating System
Sand Filtration	Rapid Mix	Sand Filter	
Coagulation	Rapid Mix	Coagulation Sedimentation	
Flotation	Rapid Mix	Flocculation/Flotation	
Solids Contact Clarifier			Solids contact clarifier
Volatilization Gas Phase Adsorption			Adsorption treatment of vapour from air stripper
Down Flow Filter			Packed PAC column
Up Flow Filter			Column of PAC attached to polystyrene spheres
Fluidized Bed			Up Flow through fluidized PAC bed
Dead End Surface Filter	Rapid Mix	Plate and frame or porous tube filter	
Crossflow Surface Filter	Rapid Mix	Ultrafiltration	
Centrifuge	Rapid Mix	Centrifuge	
Multistage Crossflow Surface Filter			Counter current flow of PAC through a series of Ultrafiltration system

TABLE 2

CRITERIA FOR EVALUATION OF REACTOR ALTERNATIVES

PART I: ESSENTIAL CRITERIA

A) Optimum Contact Time

B) Contact Stratification

C) Reliable Separation

D) Spill Response

E) Adaptable to Existing Facilities

F) Process Reliability

G) Mechanical Reliability

PART II: DESIRABLE CRITERIA

H) Portability

I) PAC Recovery

PART III: COST CRITERIA

J) Capital Cost

K) Labour

L) Operation and Maintenance

FIGURE 1 : PAC CONTACTING AND SEPARATION

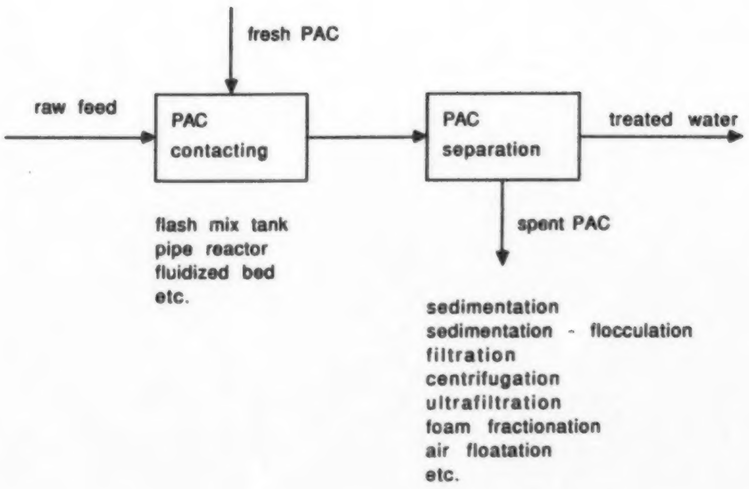

fresh PAC
raw feed
PAC contacting
PAC separation
treated water
spent PAC
flash mix tank
pipe reactor
fluidized bed
etc.
sedimentation
sedimentation - flocculation
filtration
centrifugation
ultrafiltration
foam fractionation
air floatation
etc.

FIGURE 2 : REACTOR - CLARIFIER

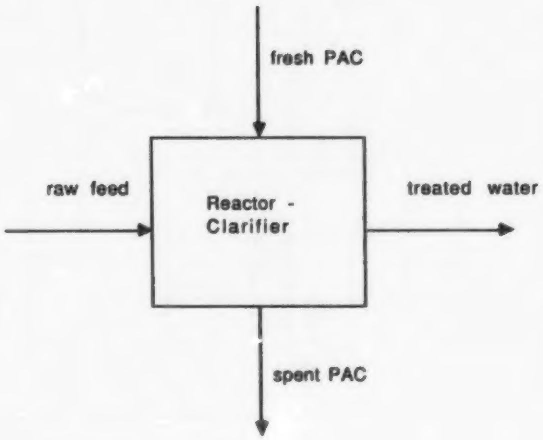
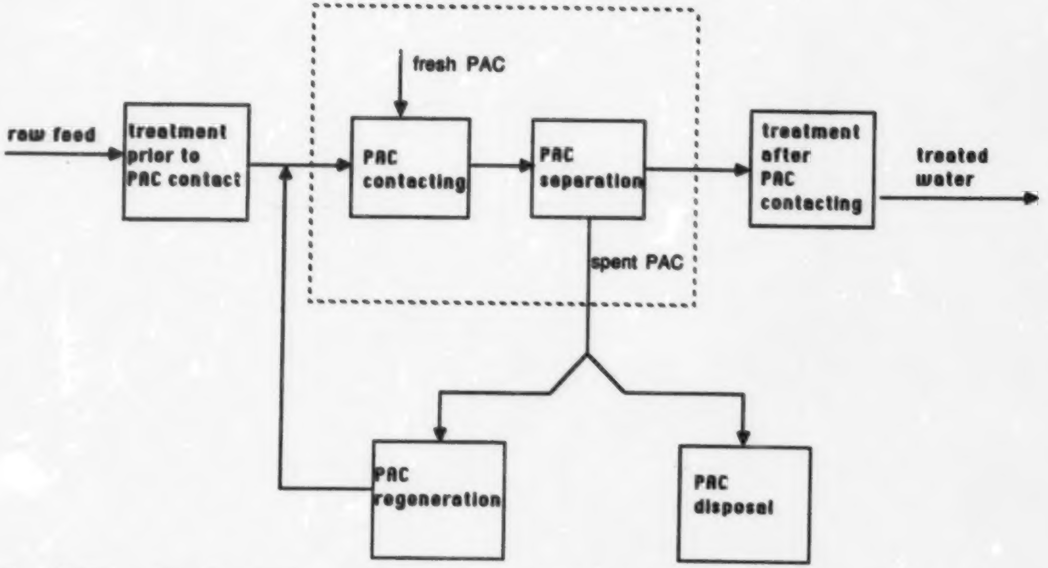

fresh PAC
raw feed
Reactor - Clarifier
treated water
spent PAC

FIGURE 3 :   OVERALL TREATMENT SCHEME


raw feed
treatment prior to PAC contact
fresh PAC
PAC contacting
PAC separation
spent PAC
treatment after PAC contacting
treated water
PAC regeneration
PAC disposal

B14

MUNICIPAL UTILIIZATION OF WATER DEMAND MANAGEMENT STRATEGIES IN ONTARIO MUNICIPALITIES

R. D. Kreutzwiser
and
R. B. Feagan

Department of Geography
University of Guelph
Guelph, Ontario, N1G 2W1

For presentation to the Environmental Research: Technology Transfer Conference, Toronto, Ontario, November 28, 1988.

INTRODUCTION

The management of municipal water supply has been dominated by a supply management philosophy, which has stressed expanding supply to meet demand (Government of Ontario, 1984). However, economic and environmental realities increasingly call into question the appropriateness of this philosophy and suggest the viability of demand management (conservation) as an alternative or supplement to traditional supply management. Recognition of this comes from senior governments in Canada (Ministry of Natural Resources et al., 1985; Canada, 1987), though present use and promotion of water conservation strategies appear limited.

This paper describes the extent of use of demand management strategies among southern Ontario municipalities, discusses several factors thought to influence municipal adoption of conservation strategies, and suggests how municipalities can enhance their conservation efforts.

METHODOLOGY

A questionnaire survey was sent to the water system managers (Public Utilities Commission general manager, city engineer or equivalent) of 315 southern Ontario municipalities with public water supply systems. The survey was designed to obtain information on use of a broad range of conservation strategies, as well as characteristics of the water system and water system manager thought to influence adoption of demand management.

Useable responses were obtained from 219 municipalities, or 70% of those surveyed. This represents a population of about 6,300,000 of the 7,000,000 people served by municipal water systems in southern Ontario.

RESULTS

Characteristics of Water Systems

An impression of the municipal water systems represented by respondents can be gained by considering the following facts. Almost 53 percent of water systems were council-run with 39 percent operated by a Public Utilities Commission. Fifty-five percent drew from a surface water source while 45 percent pumped from a groundwater source.

The emphasis on supply management is evidenced, in part, by the fact that in the last ten years, 35 percent of systems expanded pumping capacity and 30 percent expanded storage capacity. Fifty-one percent of those on groundwater added wells and 10 percent of all responding systems added treatment capacity. Problems are evident, however, in that 36 percent of systems encountered summer shortages, 36 perecent experienced water quality problems and 32 percent had major distribution leaks during the past ten years.

Utilization of Demand Management Strategies

Table 1 reports the extent of municipal utilization of a wide range of demand management strategies.

In terms of economic strategies, only three municipalities made any use of a metered inclining rate, which is considered a very powerful conserving strategy. Thirty-six percent made some use of a metered flat rate, while 40 percent used a metered decliniing rate. A set price rate structure was in place in almost 60 percent of systems. While attaching a price to water use, this strategy has no real conserving influencing. Forty-eight percent of responding municipalities imposed a sewer surcharge and 54 percent increased rates in 1987.

As reflected in Table 1, use of water metering is extensive; 79 percent of municipalities had metering in place for at least some use sectors. Almost half had some metering of residential users, and extent of metering was higher in other sectors. Only a minority of responding municipalities spent more on meter installation and repair and distribution system leak detection and repair in 1986 than the mean of their expenditures over the previous five years.

Municipal use of regulatory strategies was generally low. Thirty-five responding municipalities have imposed summer use restrictions and 22 percent have used voluntary restrictions on water use. Only 17 municipalities had water-conserving plumbing ordinances iin place.

Educational strategies were also little used, with only 22 percent of municipalities distributing educational material with water bills. Fewer (six percent) made any attempt to educate major commercial or industrial water users. Only 6 municipalities distributed retro-fit water-conserving devices in an effort to encourage water conservation among residential users.

Several water conservation strategies described in the municipal water conservation literature were not in use among any of the responding municipalities. These were daily peak-hour rate structure, marginal pricing for new water users, and limiting distribution system pressure to a minimum acceptable level.

Influences on Utilization

Several characteristics of the water system and water system manager were hypothesized as influencing municipal adoption of demand management strategies. Chi-square analysis was used to test for associations. It was found that greater use of water conservation strategies was associated with larger populations seved, older distribution system age, greater extent of water problems experienced, involvement of regional municipalities as water suppliers, and systems operated by a Public Utilities Commission. It was thought that use of water conservation strategies would be associated with municipalities drawing from a groundwater source, rather than a surface water. However, generally the opposite was so, which may reflect the increasing costs of treting surface water. No association was found between adoption of water conservation strategies and experience and training of water system managers.

Many of the factors outlined above are interrelated, and further analysis was conducted to isolate the most influential independent factors. It appears that population served and extent of problems are most strongly associated with municipal adoption of demand management strategies.

Enhancing Demand Management

Municpal water management literature suggests that demand management can reduce supply and treatment costs, reduce waste water treatment costs, off-set temporary supply shortages, reduce water appropriation conflicts, minimize environmental costs of system expansion, and defer system expansion (Ellis, 1978; Maier et al., 1981; Sawyer, 1983; Grima, 1985). Despite these reported benefits, municipal adoption of the demand management concept among southern Ontario municipalities has not been extensive.

There are, however, several actions municipalities can take to enhance water conservation. Municipalities, for example, can move toward full metering of all water users. While it can be debated whether meterinig by itself has a major conserving influence, users become aware of the volume used and they pay in proportion to use. Loudon (1984) found a 13-20 percent decline inw ater use in Durham Region after introduction of metering. Metering is also a basis for applying rate structures that can influence use further.

Municipalities can also move away from a declining rate structure to more conserving structures. While the flat metered rate is less effective than the inclining metered rate, it is more equitable and implementable. General rate increases, to better reflect the full costs of water supply and the value of the resource, can also influence water use. Several studies, for example Ellis (1978), suggested an average absolute price elasticity for municipal water of about .5, which means that a price increase of ten percent would result in a five percent reduction in use.

Municipalities can increase effort to detect and repair distribution system leaks. MacLaren (1985) estimated 28 percent unaccounted for use among Ontario municipalities and 25 breaks per 100 km per year. Under these circumstances, leak detection and repair could be cost-effective.

Barclay (1984) suggested that water-conserving devices on plumbing fixtures can reduce water use by over 30 percent. Plumbing and building ordinances, though presently little used in Ontario, offer the potential to reduce consumption substantially. Limiting distribution system pressure to minimum levels acceptable for fire-fighting purposes can also reduce use and leakage, though this reduction may not be great.

Education is usually considered to have minimal impact on water use behaviour, while economic strategies appear to offer greatest potential. However, education can have an important role in enhancing the local acceptability and adoption of more effective strategies. Edcucation can also play a role in developing political support for changes in senior government grant structures and other arrangements influencing local decision-making, to ensure that appropriate incentives for demand management are established.

ACKNOWLEDGEMENTS

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Table 1 Use of Water Conservation Strategies Among Southern Ontario Municipalities

Strategy	Percent of Municipalities
Rate structure	
- set price	58.9
- metered declining	40.2
- metered flat	36.1
- metered inclining	1.4
Overall rate increase (1987)	42.9
Sewer surcharge	64.8
Metering	
- any sector	78.9
- residential	49.3
- commercial	68.9
- industrial	60.3
- institutional	52.9
1986 Metering budget greater than mean of previous 5 years	11.9
1986 Distribution system leak budget greater than mean of previous 5 years	13.7
Summer use restrictions	34.7
Voluntary restrictions	21.6
Plumbing and building ordinances	7.8
Water-conserving devices	2.7
Educational initiatives	
- pamphlets	21.5
- media information	12.8
- treatment plant tours	17.4
- commercial/industrial programs	6.4

B15

A Preliminary Study to Determine the Feasibility of Medium Pressure Mercury Lamps for Disinfecting Low Quality Wastewaters.

G.E. Whitby[1], J. Maarschalkerweerd[1], and
G.A. Palmateer[2]

1. Trojan Technologies Inc.
845 Consortium Court
London, Ontario
N6E 2S8

2. Ministry of the Environment
985 Adelaide Street South
London, Ontario
N6E 1V3

INTRODUCTION

During dry weather most wastewaters receive some form of chemical or biological treatment to remove organic and inorganic constituents and then these effluents are disinfected to protect users of the receiving waters. It is only during periods of rainfall that significant quantities of effluents such as stormwater runoff and combined sewer overflow are allowed to flow into watercourses with little or no treatment. Combined sewer overflow is the result of joint wastewater and surface runoff collection systems. These combined sewer systems are prevalent in older areas of most municipalities. These microbiologically contaminated waters have contaminated raw water supplies and swimming areas throughout North America. The closure of swimming areas is a great inconvenience for everyone and results in a loss of revenue for those involved in tourism.

Programs to alleviate the situation include the separation of combined sewers which is very expensive, methods of reducing the volume and frequency of overflows, and methods of improving the quality of the storm runoff. It is this latter aspect where the use of medium pressure mercury ultraviolet lamps was investigated for the purpose of disinfecting low quality wastewaters.

Previous studies (Scheible, 1985 and Zukovs, et al. 1986) have shown that combined sewer overflow (CSO) can be disinfected with low pressure mercury lamps but the capital cost of the system is very high compared to chlorine because of the large number of lamps. The high cost is a result of the high flow rates and long retention times which are required to obtain a three logarithm reduction in the number of fecal coliforms.

A medium pressure mercury lamp has a much higher intensity per unit of arc length compared to a low pressure mercury lamp. A low pressure mercury lamp which is normally used for the disinfection of liquids has a UV output of approximately 0.2 watts per centimeter of arc length at a wavelength of 254 nm. The output of UV light is almost monochromatic and within six nanometres of the optimum wavelength for germicidal action. Medium pressure mercury lamps have an average UV output of 9 watts per centimeter of arc length at wavelengths below 380 nm.

If all of these wavelengths below 380 nm were equally effective at killing fecal coliforms, a medium pressure mercury lamp would have 45 times as much germicidal power per centimeter of arc length. A significant decrease in the number of UV lamps would result in a much lower capital cost which could make UV irradiation of low quality wastewaters an economically viable process.

The study had three phases. The first phase determined the dose of UV light which was required from a low and medium pressure mercury lamp to disinfect a series of different quality wastewaters. The second phase looked at the total UV output of low and medium pressure mercury lamp so that a pilot system could be built. The third phase involved the testing of a medium pressure lamp system with three different waterlayers to determine the economic feasibility of the process.

PHASE 1: DIRECT COMPARISON OF THE LOW AND MEDIUM PRESSURE MERCURY LAMPS WITH THE LOW QUALITY WASTEWATERS.

1. Purpose

This phase of the study compared the monochromatic light (254 nm wavelength) of the low pressure mercury lamp to the broader spectrum of the medium pressure mercury lamp. The bioassay method of Qualls and Johnson (1983) was used to make this comparison. The bioassay uses a collimated beam of light to irradiate a volume of stirred wastewater.

Two collimated beams were adjusted so that a UV sensor which was only sensitive to light around a wavelength of 254 nm showed that the beams were equal in UV output. The additional wavelengths in the spectrum of the medium pressure lamp should show up in the survival curves of the fecal coliforms in the various wastewaters. These survival curves can then be used to compare the two types of mercury lamps. The dose of UV light was adjusted so that a three logarithm kill or 200 fecal coliforms per 100 mL was reached in every wastewater.

2. Materials and Methods

Samples of raw wastewater after the comminuting devices, raw wastewater after primary settling and secondary treated wastewater were obtained from the Greenway Wastewater Treatment Plant in London, Ontario, Canada. Samples of raw water after the comminuting devices were also obtained from the wastewater treatment plant in Ingersoll, Ontario, Canada.

Various strengths of CSO were prepared by mixing secondarily treated wastewater so it contained 13.5, 25 and 50 percent raw effluent.

The bioassay method of Qualls and Johnson (1983) was modified so that the medium and low pressure mercury lamps could be compared. Two collimated beams (Figure 1) were set up side by side so that the same day's samples was irradiated with the medium and low pressure mercury lamps.

Both light sources were set at 200 microwatts/cm² at the liquid surface with an International Light 1500 Radiometer with an SEE 240 sensor (International Light Inc. Dexter Industrial Green, Newburyport, Massachusetts, USA).

A single (50mL) of wastewater was measured into the irradiation chamber shown in Figure 1 and continuously stirred during the exposure to UV light. A series of exposure times was used for each wastewater. The wastewater was 2 centimeters deep.

Unirradiated samples were stirred to determine whether the suspended solids were being broken up by the magnetic stirring bar.

Each sample of wastewater was analyzed for UV transmittance at a wavelength of 254 nm. Each wastewater was filtered through a 0.45 micron Gelman GN type filter and analyzed for UV transmittance at a wavelength of 254 nm.

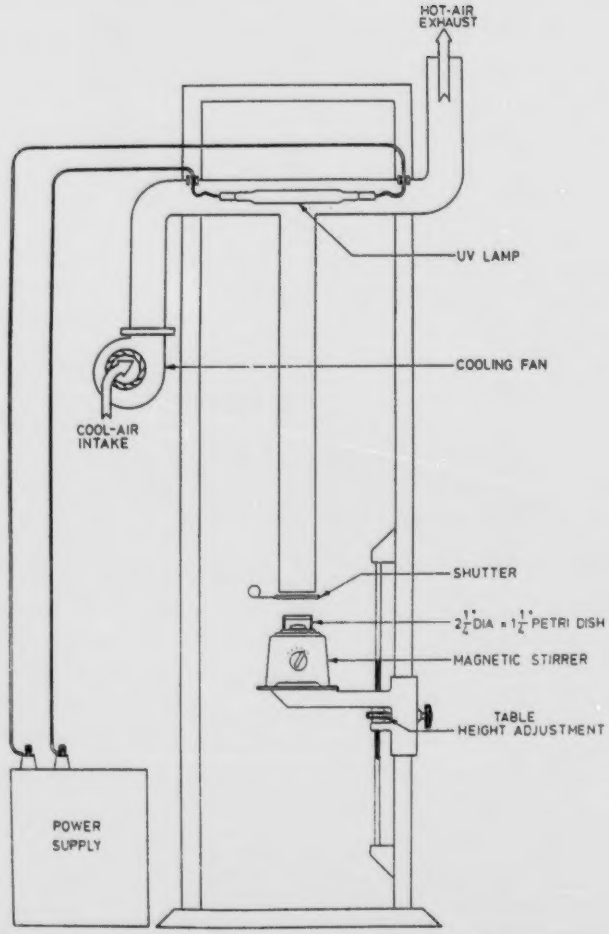
HOT-AIR EXHAUST
UV LAMP
COOLING FAN
COOL-AIR INTAKE
SHUTTER
$2\frac{1}{2}$" DIA × $1\frac{1}{2}$" PETRI DISH
MAGNETIC STIRRER
TABLE HEIGHT ADJUSTMENT
POWER SUPPLY

Figure 1: Schematic diagram of the collimated beam apparatus for irradiating the various wastewaters.

The total suspended solids of each wastewater was analyzed according to Method 209C in the 16th Edition of Standard Methods For The Examination of Water and Wastewater (American Public Health Association, 1985).

The fecal coliforms were measured by the membrane filtration and rota-plate method. The membrane filtration method and the media for the rota-plate method were from the (Ontario Ministry of the Environment, 1984) Handbook of Analytical Methods for Environmental Samples.

Each wastewater was tested at least five times or until consistent results were obtained.

3. Results and Discussion

The results in Table 1 show that stirring the raw, primary and secondarily treated wastewaters in the irradiation chambers had no effect on the count of the fecal coliforms. Therefore, the samples can be stirred during the irradiation without breaking up the suspended solids. This is important because the size and level of suspended solids affects the degree of disinfection which can be attained (Qualls *et al*., 1985).

Figure 2 to 8 and Table 2 summarize the paired testing of the low and medium pressure mercury lamps on the raw, primary, secondary and mixtures of wastewaters.

All of the kill curves are typical of that found for fecal coliforms in wastewater (Qualls *et al*., 1985) in that a final plateau is reached where increases in the dose of UV light has very little effect on the level of fecal coliforms. This is due to the suspended solids which protect the fecal coliforms from the UV irradiation.

Table 1: The effect of stirring the wastewater in the irradiation chambers on the level of fecal coliforms.

Wastewater	Geometric Mean Fecal Coliforms per 100 ml Time in Minutes 0	 40
Raw	1.2×10^6	1.3×10^6
Primary	1.4×10^6	1.5×10^6
Secondary	1.4×10^4	1.4×10^4

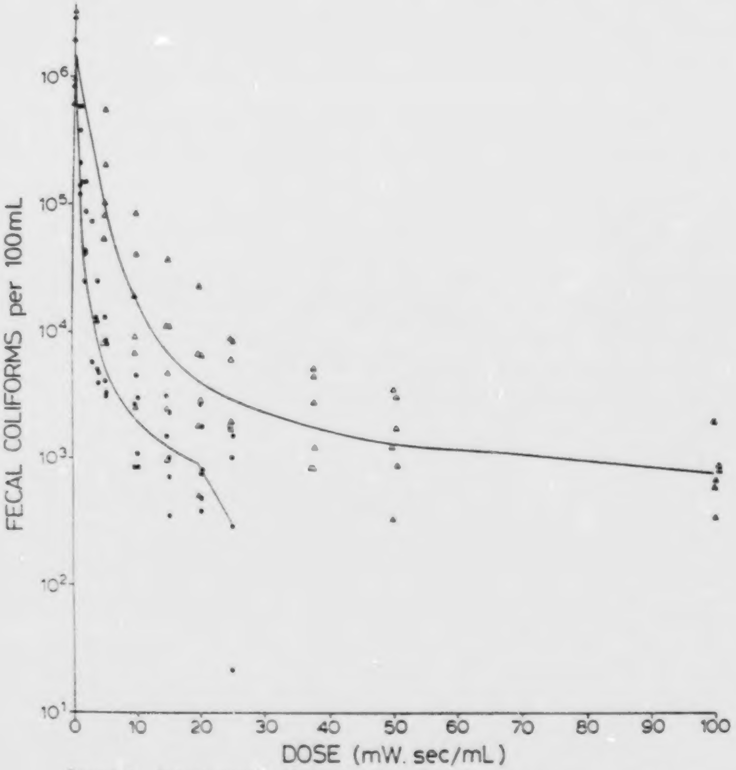

Figure 2: Paired testing of the low (Δ) and medium (•) pressure mercury lamps on the raw effluent from the Greenway Wastewater Treatment Plant using the collimated beam.

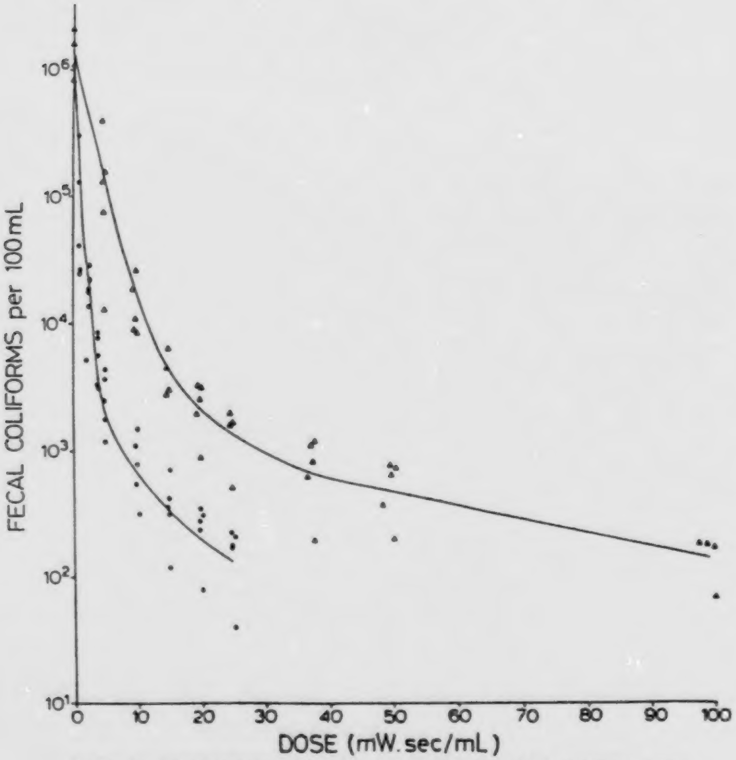

Figure 3: Paired testing of the low (Δ) and medium (•) pressure mercury lamps on the primary effluent from the Greenway wastewater treatment plant using the collimated beam.

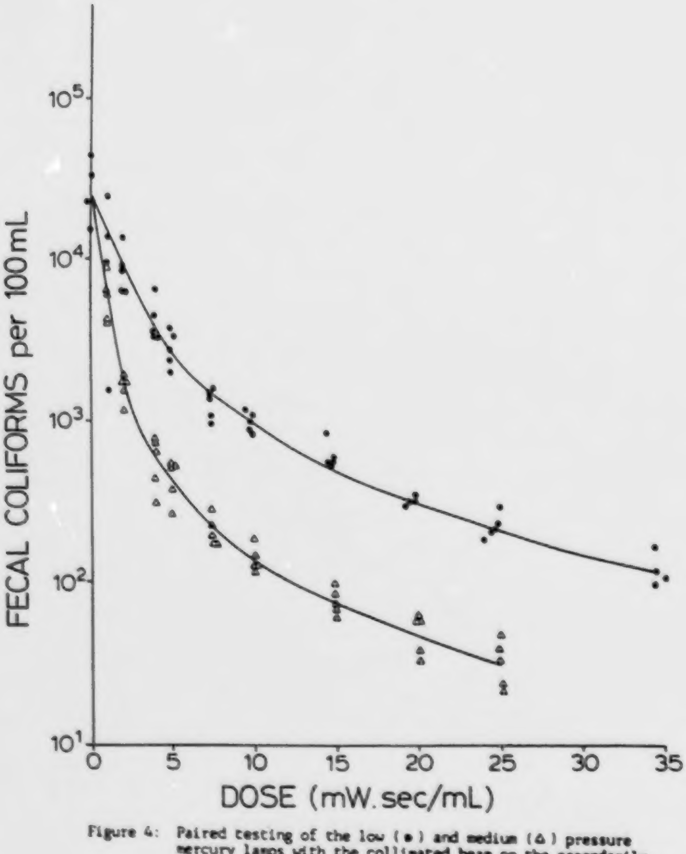

Figure 4: Paired testing of the low (●) and medium (△) pressure mercury lamps with the collimated beam on the secondarily treated effluent from the Greenway wastewater treatment plant.

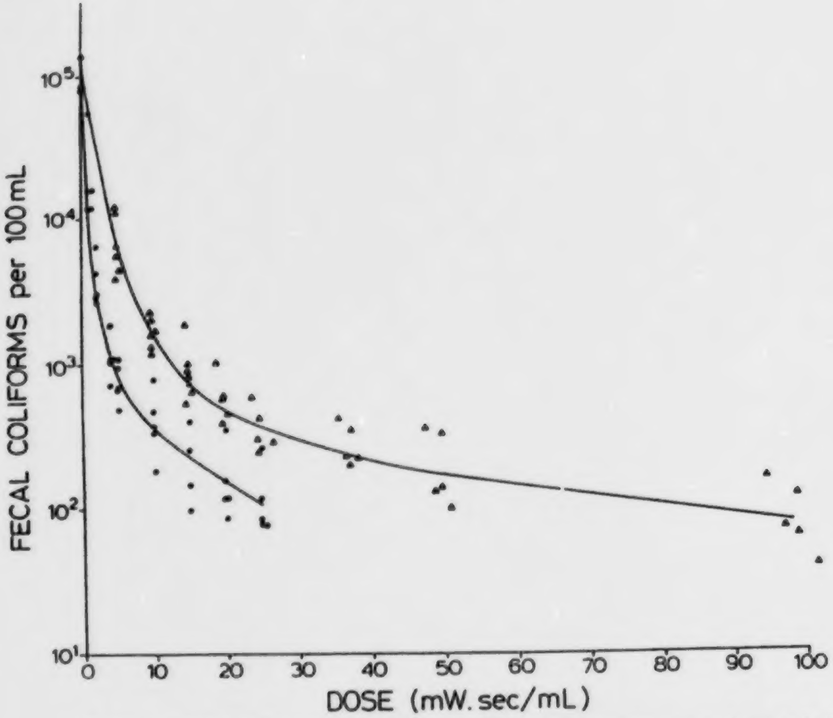

Figure 5: Paired testing of the low (▵) and medium (•) pressure mercury lamps with the collimated beam on the mixture of 12.5% raw effluent and 87.5% secondary effluent from the Greenway wastewater treatment plant.

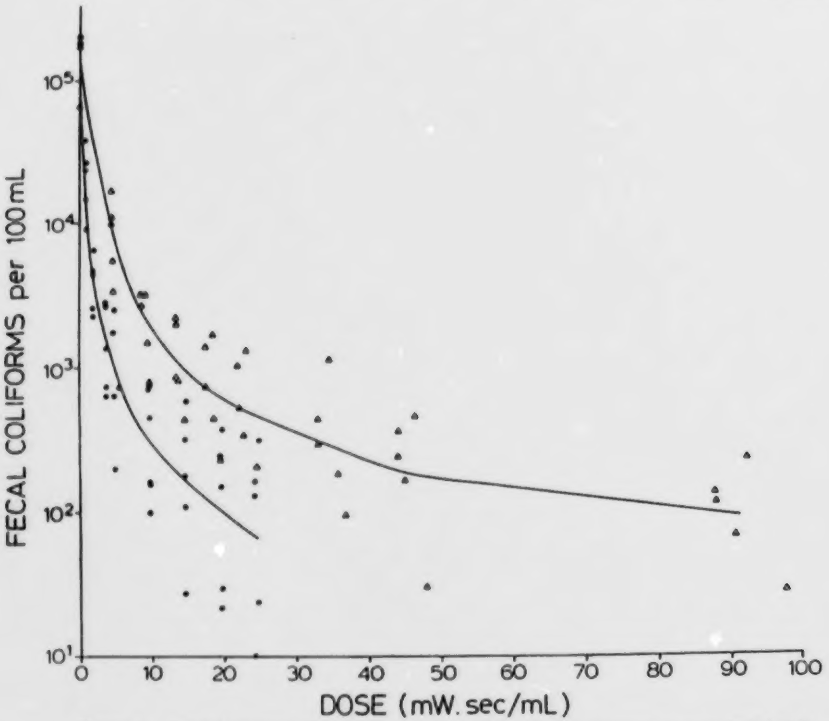

Figure 6: Paired testing of the low (Δ) and medium (•) pressure mercury lamps with the collimated beam on the mixture of 25% raw and 75% secondary effluent from the Greenway wastewater treatment plant.

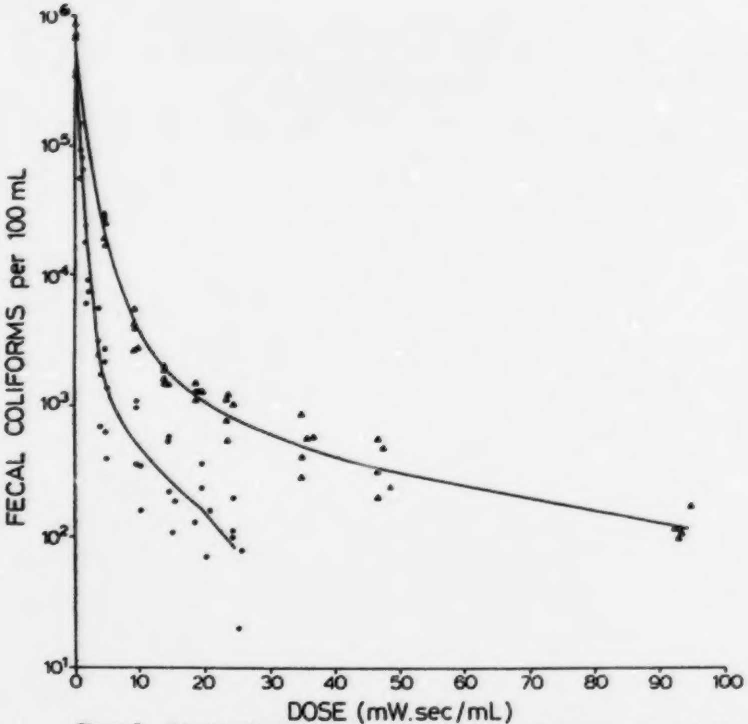

Figure 7: Paired testing of the low (Δ) and medium (●) pressure mercury lamps with the collimated beam on the mixture of 50% raw and 50% secondary effluent from the Greenway wastewater treatment plant.

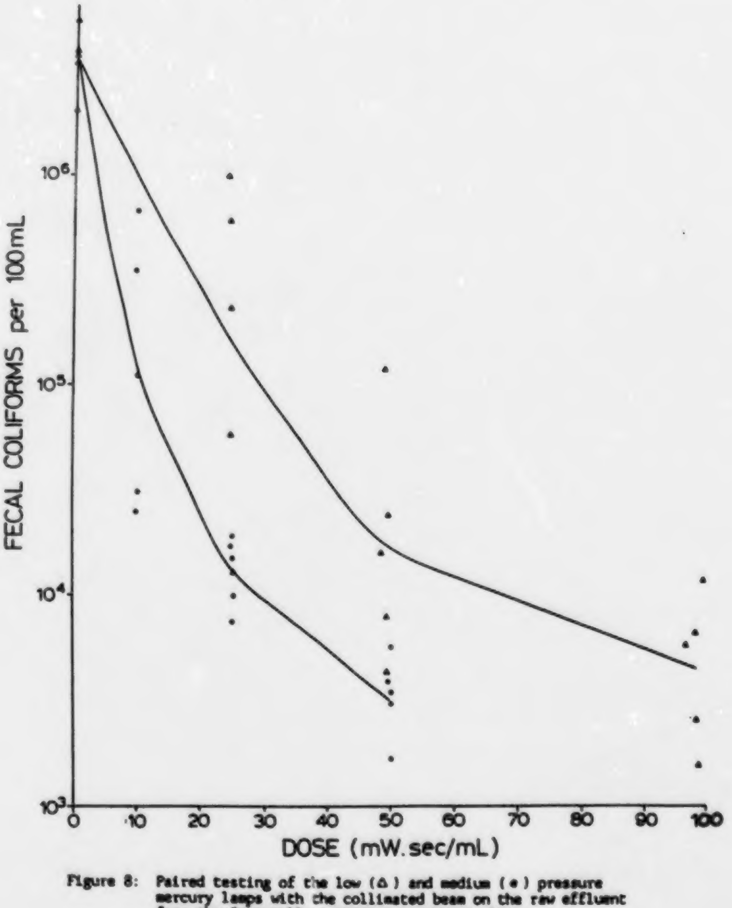

Figure 8: Paired testing of the low (Δ) and medium (•) pressure mercury lamps with the collimated beam on the raw effluent from the Ingersoll wastewater treatment plant.

Table 2: The filtered and unfiltered UV transmittance and total suspended solids of the various wastewaters and mixtures of wastewaters.

Wastewater		% Transmission $^{254}_{1\ cm}$ Unfiltered	Filtered	Total Solids (mg/L)
Raw	Mean	27.7	63.7	121
	SD*	2.5	3.9	28
Primary	Mean	30.0	62.9	62
	SD	4.6	3.6	15
Secondary	Mean	71.7	77.5	13.4
	SD	0.2	0.5	0.9
12.5% Raw: 87.5% Secondary	Mean	58.9	74.1	30
	SD	2.8	2.5	10
25% Raw: 75% Secondary	Mean	57.9	75.8	35
	SD	7.8	1.3	18
50% Raw: 50% Secondary	Mean	49.2	72.5	31
	SD	7.2	3.7	10
Raw from Ingersoll	Mean	1.5	34.4	411
	SD	1.1	7.5	169

In these experiments, the maximum dose of UV light from the low and medium pressure mercury lamps was able to reduce the count of fecal coliforms in the primary, secondary mixtures of wastewaters to below 200 fecal coliforms per 100 mL. The level of fecal coliforms in the raw effluents from the two wastewater treatment plants could not be reduced to 200 fecal coliforms per 100 mL. The high suspended solids (411 mg/L) and low UV transmission (1.1%) of the raw wastewater from Ingersoll accounts for this difference. The suspended solids accounts for this difference with the raw wastewater from Greenway because the UV transmission of the filtered and unfiltered samples were almost identical for the raw and primary effluents.

As the wastewater proceeds through the Greenway wastewater treatment plant, the unfiltered UV transmission increases by 2.6 times whereas the filtered effluent only increases by 1.2 times. The level of suspended solids decreases nine fold as the wastewater goes from the raw water to the end of the secondary treatment. Therefore the increase in UV transmission is primarily due to the decrease in suspended solids.

Curves of the survival ratio versus the dose are shown in Figures 9 to 15 for the various wastewaters. From these curves and Figures 2 to 8, the dose of UV light can be obtained which produces a three log decrease in the count of the fecal coliforms or reduces the level of fecal coliforms to 200 per 100 mL. These doses are summarized in Table 3. This table shows the quantity of UV light from a medium or low pressure mercury lamp which is required to bring the level of fecal coliforms in a volume of one millilitre down by three logs or to 200 per 100 millilitres. The dose of UV light varies quite dramatically between the different wastewaters. This is a result of the differences in the level and form of the suspended solids and the UV transmission. The flow rate can be calculated using these values for the dose and knowing the total UV output of the lamp. The total UV output of the two types of lamps was determined in Phase 2.

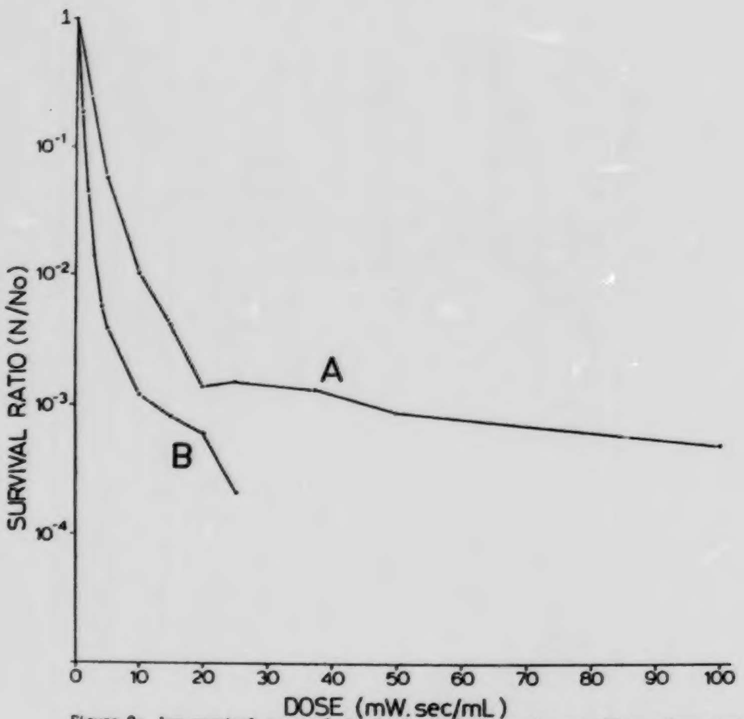

Figure 9: Log survival curve of the fecal coliforms in the raw effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

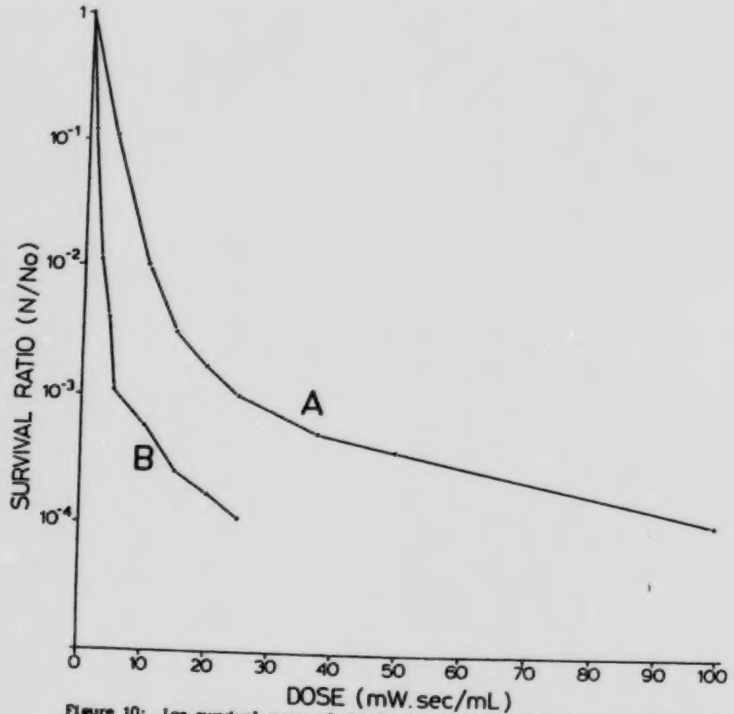

Figure 10: Log survival curve of the fecal coliforms in the primary effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

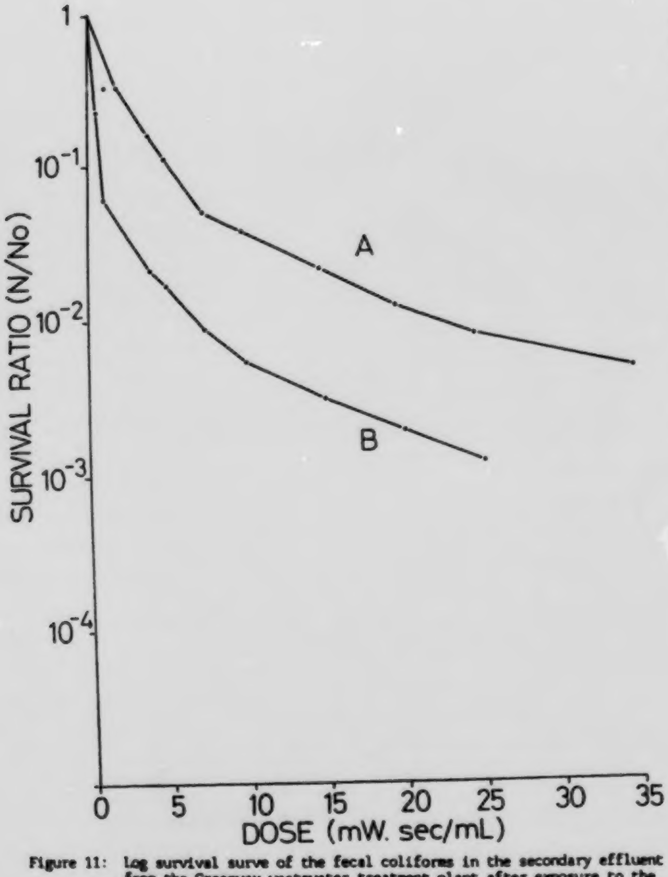

Figure 11: Log survival surve of the fecal coliforms in the secondary effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

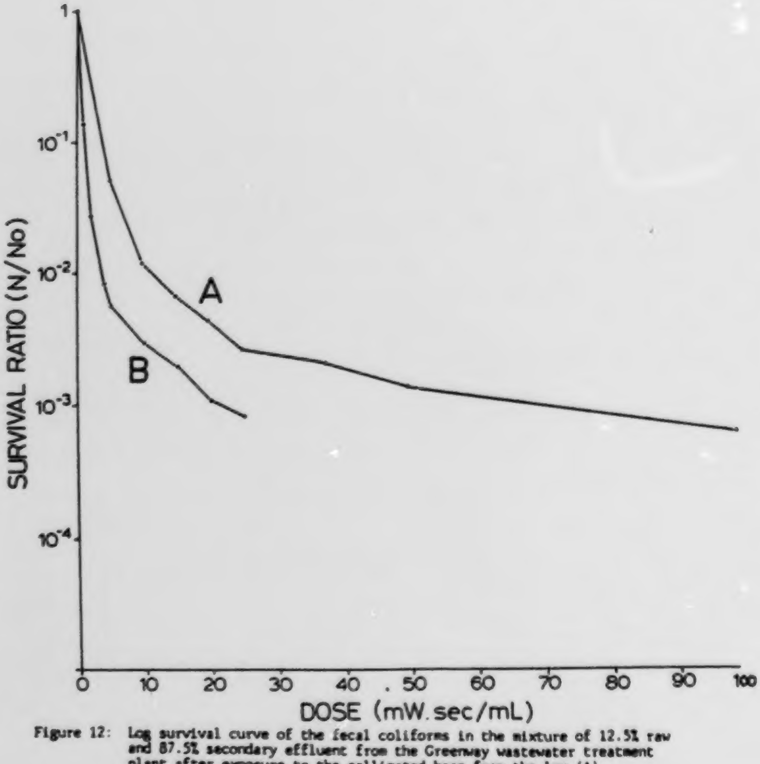

Figure 12: Log survival curve of the fecal coliforms in the mixture of 12.5% raw and 87.5% secondary effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

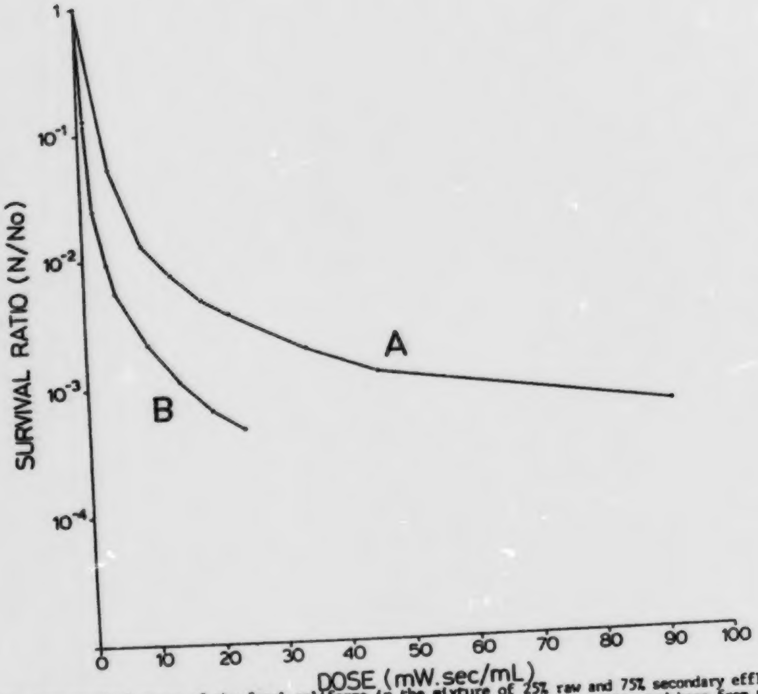

Figure 13: Log survival curve of the fecal coliforms in the mixture of 25% raw and 75% secondary effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

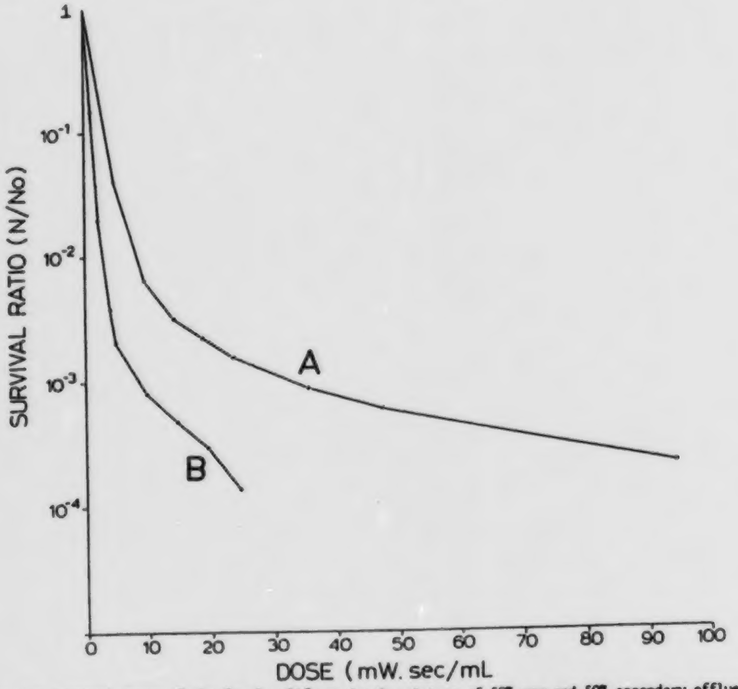

Figure 14: Log survival curve of the fecal coliforms in the mixture of 50% raw and 50% secondary effluent from the Greenway wastewater treatment plant after exposure to the collimated beam from the low (A) and medium (B) pressure mercury lamps.

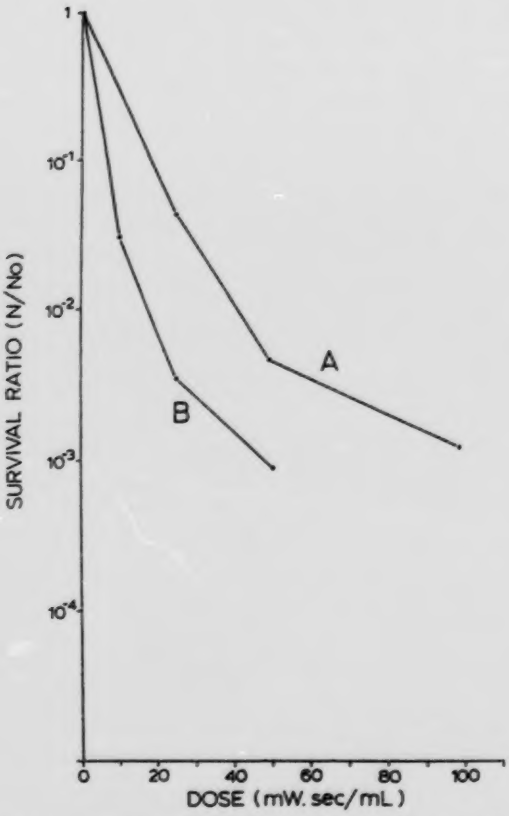

Figure 15: Log survival curve of the fecal coliforms in the raw effluent from the Ingersoll wastewater treatment plant after exposure to the low (A) and medium (B) pressure mercury lamps.

Table 3: The dose of UV light from the medium or low pressure mercury lamp which produces a three log decrease in the count of fecal coliforms or reduces the count of the fecal coliforms to 200 per 100 ml.

Wastewater	Fecal Coliform Limit	Dose (mW.sec.mL^{-1}) Medium Pressure Lamp	Low Pressure Lamp
Raw	3 Log Decrease	13	41
	200 per 100 mL	Not Reached	
Primary	3 Log Decrease	6	25
	200 per 100 mL	21	85
Secondary	3 Log Decrease	Not Reached	
	200 per 100 mL	6	25
12.5% Raw:87.5% Secondary	3 Log Decrease	22	69
	200 per 100mL	27	83
25% Raw: 75% Secondary	3 Log Decrease	16	56
	200 per 100 mL	14	43
50% Raw: 50% Secondary	3 Log Decrease	9	32
	200 per 100 mL	18	71
Raw Ingersoll	3 Log Decrease	49	102
	200 per 100mL	Not Reached	

The ratios of the dose of UV light from the low and medium pressure lamps for the various wastewaters are shown in Table 4. For the Greenway wastewaters and mixtures of wastewaters the ratio is 3.6 (Standard Deviation = 0.5) and it is consistent between the various types of wastewaters whereas the ratio for the raw effluent from Ingersoll is 2.1 Because the medium pressure mercury lamp has a much broader spectrum of germicidal light, this ratio may be very specific for each effluent depending upon its absorption spectrum. The raw effluent from Ingersoll has a very high iron content due to the presence of a wire manufacturer. Iron readily absorbs UV light from the low and medium pressure mercury lamps.

4. Conclusions

1. Stirring the irradiation chambers had no effect on the level of the fecal coliforms in the raw, primary or secondary effluent from the Greenway wastewater treatment plants. Suspended solids were not being broken up so the results of the irradiation should not be influenced by the release of fecal coliforms from particles.

2. Suspended solids in the Greenway effluents have the greatest effect on changes in the UV transmission as the wastewater proceeds through the plant.

3. Each type of wastewater requires a different dose of UV light to reach the required level of disinfection. This is a result of the UV transmission and suspended solids and the relationship of the fecal coliforms with these particles. To properly size a UV system for CSO, a series of survival curves should be prepared using the method described in this report.

4. When each watt of UV light from a medium pressure mercury lamp was measured with an IL 1500 Radiometer with a SEE240 sensor, it was equivalent to 3.6 watts of UV light at a wavelength of 254 nm from a low pressure mercury lamp when the wastewater was from the Greenway Wastewater Treatment Plant.

Table 4: The ratio of the doses of UV light from the low and medium pressure mercury lamps which were required to reach a three log decrease or 200 fecal coliforms per 100 mL.

	Ratio of Dose $\frac{\text{Low Pressure Lamp}}{\text{Medium Pressure Lamp}}$	
Wastewater	Three Log Decrease in Fecal Coliforms	200 Fecal Coliforms per 100mL
Raw	3.1	-
Primary	4.2	4.0
Secondary	-	4.2
12.5% Raw: 87.5% Secondary	3.1	3.1
25% Raw: 75% Secondary	3.5	3.1
50% Raw: 50% Secondary	3.5	3.9
Raw Ingersoll	2.1	-

PHASE 2: TOTAL GERMICIDAL OUTPUT OF THE LOW AND MEDIUM PRESSURE MERCURY LAMPS

1. Purpose

The objective of this phase of the project was to determine and compare the total germicidal output of the low and medium pressure lamps. This would allow an economic comparison of the two lamps and it will also provide the information which is required for the design of the medium pressure reactor vessel in Phase 3.

2. Materials and Methods

The point source summation method of Qualls and Johnson (1983) was used to determine the UV output of the low and medium pressure mercury lamps. The intensity was measured at 150 cm from the centroid of the lamps.

The low pressure mercury lamps were from Voltarc Tubes Inc., type G36T6L. Three of these lamps were burned for 100 hours before measurements were taken.

One of the 2000 Watt medium pressure mercury lamps were from Voltarc Tubes Inc. Two of the 2000 Watt medium pressure mercury lamps were from W.C. Heraeus.

3. Results and Discussion

The UV light output of the low and medium pressure mercury lamps are summarized in Table 5. The average output of UV light by the 2000 W medium pressure mercury lamps as measured by the point source summation method with the SEE240 sensor is 52.2 watts (Standard Deviation = 1.9). This can be converted to the germicidal power of the low pressure mercury lamp by multiplying by 3.6. The results from Phase 1 showed that each watt of UV light from the medium pressure mercury lamp was equivalent to 3.6 watts of UV light from a low pressure mercury lamp when wastewater from Greenway was being disinfected. The 2000 watt medium pressure mercury lamp has the equivalent of 188 watts of UV light from a low pressure mercury lamp.

The average output of UV light from the low pressure mercury lamps (G36T6L) after 100 hours of burning was 13.2 watts (Standard Deviation = 0.5) as measured by the point source summation method.

One - 2000 watt medium pressure mercury lamp is equivalent to 14.2 low pressure mercury lamps (G36T6L) when effluent from Greenway was being disinfected.

The total output of the two types of lamps as measured by the point source summation method can be used to calculate the flow rates for the various wastewaters. These flow rates are obtained by dividing the total UV output by the dose of UV light required per millilitre of effluent and then converting the answer to litres per minute.

Table 5: UV light output of the medium and low pressure mercury lamps as measured by the point source summation method.

Lamp Type	Watts
Medium Pressure	
Heraeus 1	52.6
Heraeus 2	53.9
Voltarc	50.1
Low Pressure	
G36T6L # 1	12.6
# 2	13.6
# 3	13.5

The flow rates are shown in Table 6 for the various wastewaters. From this Table it can be seen that the flow rates are extremely variable between the various wastewaters. This is a result of the variations in suspended solids, UV transmission and the initial numbers of fecal coliforms. This data on flow rates for a 2000 watt medium pressure mercury lamp will be used to build a UV unit with three different water layers. This UV unit is described in Phase 3. This UV unit will be tested with raw effluent at flow rates of 482, 241 and 120 litres per minute and with primary effluent at flow rates of 1044, 522 and 261 litres per minute.

4. Conclusions

1. Using the point source summation method, the 2000 watt medium pressure mercury lamp produced 52.2 watts of UV light at 254 nm and the low pressure mercury G36T6L lamp put out 13.2 watts of UV light at the same wavelength.

2. The flow rates for the various wastewaters are quite variable due to the differences in suspended solids, UV transmission and numbers of fecal coliforms. Each wastewater should be characterized before being treated with UV light. This characterization should include: suspended solids, UV transmission, level of fecal coliforms and a survival curve of the fecal coliforms.

Table 6: The flow rates of the low and medium pressure lamps for the various wastewaters

Wastewater	Fecal Coliform Limit	Flow Rate (L/min)	
		2000W Medium Pressure	G36T6L Low Pressure
Raw	3 Log Decrease	241	19
	200 per 100 mL	Not Reached	
Primary	3 Log Decrease	522	32
	200 per 100 mL	149	9
Secondary	3 Log Decrease	Not Reached	
	200 per 100 mL	522	32
12.5% Raw: 87.5% Secondary	3 Log Decrease	142	11
	200 per 100 mL	116	9
25% Raw: 75% Secondary	3 Log Decrease	196	14
	200 per 100 mL	224	18
50% Raw: 50% Secondary	3 Log Decrease	348	25
	200 per 100 mL	174	11
Raw Ingersoll	3 Log Decrease	64	8
	200 per 100 mL	Not Reached	

PHASE 3: CONTINUOUS FLOW TESTING OF THE MEDIUM PRESSURE MERCURY LAMP

1. Introduction

Liquids with a low UV transmission and/or high suspended solids must be thoroughly mixed as they pass through a UV unit so that each microorganism is subjected to a maximum dose of UV light. Table 2 shows that raw and primary effluent have a low UV transmission and high suspended solids compared to secondarily treated wastewater. A UV unit containing a 2000 watt medium pressure lamp was built with three different water layers to determine the effect of water depth and flow rate on the disinfection of raw and primary wastewater.

2. Design Specifications and Rationale for the Continuous Flow Reactor

Of the wastewaters examined during Phase 1 of this study, two were chosen for the continuous flow study and these were primary and raw effluent from Greenway Wastewater Treatment Plant in London, Ontario, Canada. These effluents would represent low quality wastewaters with and without sedimentation.

The proposal for the project called for mechanical mixing of the wastewater as it passed by the UV lamp but because of the high flow rates this was substituted with three different water layers around the UV lamp. The UV unit was built with a long inlet and smooth surfaces and curves to minimize the dead corners as the wastewater passed from one water layer to the next. The three water layers were built as one unit to minimize the effect of having three different UV units. The water layers could be studied at the same flow rate in rapid succession to reduce the effect of changes in the wastewater quality.

The UV unit was built around the 2000 watt medium pressure mercury lamp which was used in Phase 1 of this study. This eliminated any changes in the lamp from one phase of the study to another. The UV system was built so that the lamp could be quickly moved without being shut off from one water layer to the next to minimize changes in the effluent and flow rates.

Phase 2 showed that the design flow rate for the raw effluent was 241 litres per minute and for the primary effluent it was 522 litres per minute. This was equivalent to a UV dose of 13mW. sec/mL for the raw effluent and 6mW. sec/mL for the primary effluent.

3. Materials and Methods

A UV unit (Figure 16) was built with three different water layers and a movable 2000 watt medium pressure mercury lamp.

Raw or primary effluent was pumped from the Greenway Wastewater Treatment Plant in London, Ontario, Canada through the UV unit at a the flow rates shown in Figures 17-28. These flow rates varied from 110 L/min to 796 L/min. The average flow rate was kept as close as possible to the optimum flow rate shown in Table 6 for raw and primary effluent. The flow rate was measured with a stopwatch and 400 litre reservoir.

The UV unit was operated in a vertical position and the effluent was pumped into the bottom of the system. After the initial minimum flow rate was set, the lamp was turned on and allowed to burn until the amperage of the lamp stabilized.

The lamp was moved by pulling a marked wire which protruded from top and bottom of the UV unit. The marked wire positioned the UV lamp in the middle of each water layer.

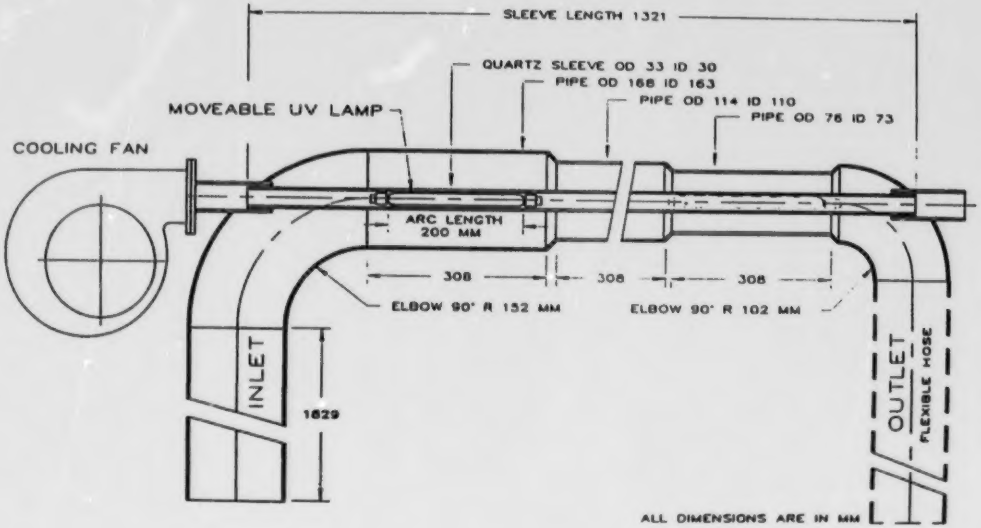
SLEEVE LENGTH 1321
QUARTZ SLEEVE OD 33 ID 30
PIPE OD 168 ID 163
PIPE OD 114 ID 110
PIPE OD 76 ID 73
MOVEABLE UV LAMP
COOLING FAN
ARC LENGTH 200 MM
308
308
308
ELBOW 90° R 152 MM
ELBOW 90° R 102 MM
INLET
1829
OUTLET
FLEXIBLE HOSE
ALL DIMENSIONS ARE IN MM

Figure 16: A schematic diagram of the continuous flow UV unit with three different water layers and a moveable UV lamp.

At the beginning of each day's run, a 3.25 kg bottle of analytical reagent grade nitric or hydrochloric acid (British Drug House, Toronto, Canada) was used to clean the quartz sleeve. The acid and water were poured into the UV unit until the quartz sleeve was submerged and then the UV system was gently rocked to mix the acid and the water. A visual inspection showed that this procedure cleaned the quartz sleeve.

Each water layer was tested at one flow rate and then the UV lamp was turned off and the flow rate changed.

Two samples were collected at each flow rate for every water layer. One was immediately put on ice in the dark and the second sample was subjected to sunlight according to the method of Whitby et al. (1984).

Each sample was analyzed for UV transmittance at a wavelength of 254 nm.

The total suspended solids of each wastewater was analyzed according to Method 209C in the 16th Edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1985).

The fecal coliforms were measured by the membrane filtration method (Ontario Ministry of the Environment, 1984).

4. Results and Discussion

a. Raw Effluent

The level and fraction survival of the fecal coliforms in the raw effluent after irradiation with the 2000 watt medium pressure mercury lamps, three different water layers and various flow rates are shown in Figures 17-22. Each Figure shows the level or fraction survival of the fecal coliforms before and after three hours in the sunlight.

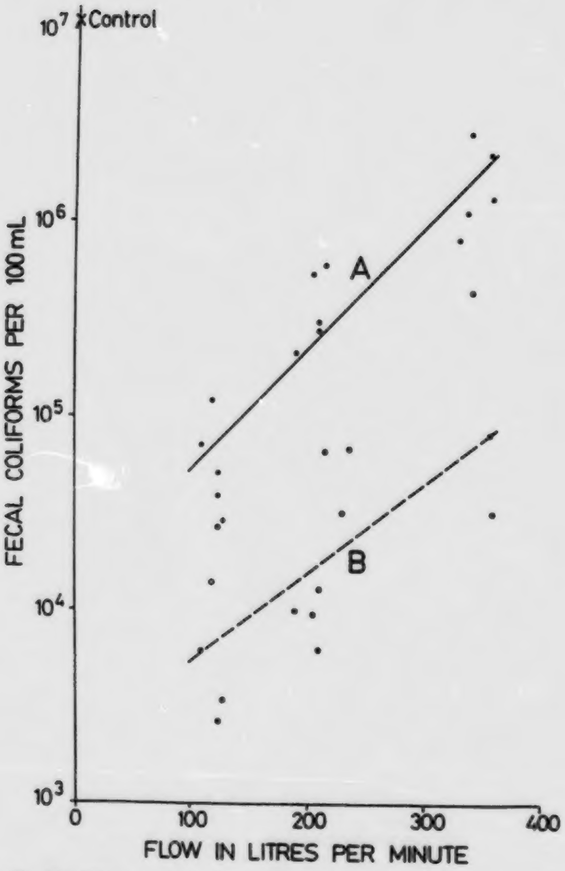

Figure 17: The effect of the two centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

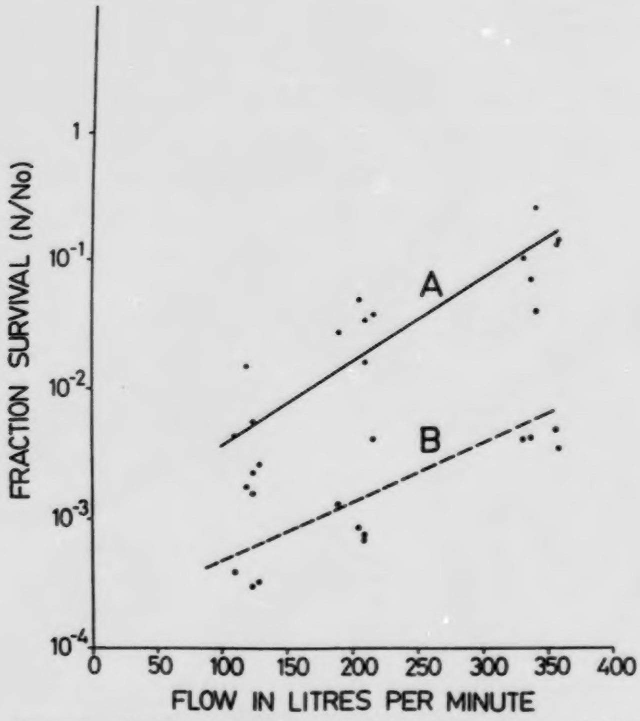

Figure 18: The effect of the two centimetre water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

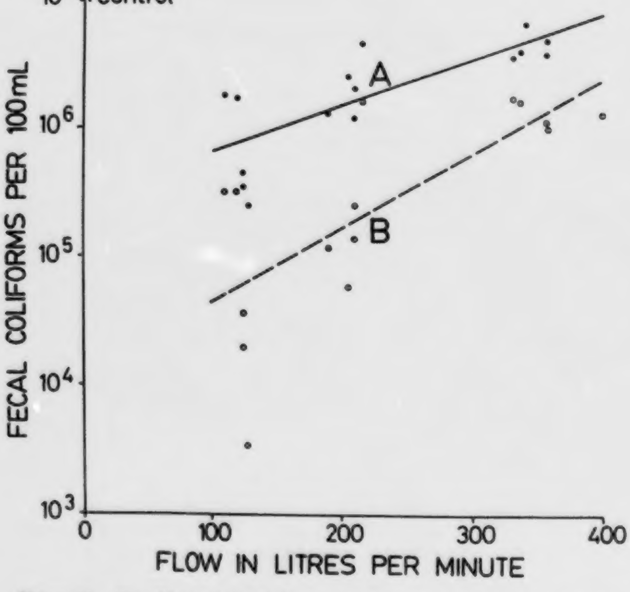

Figure 19: The effect of the 3.85 centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

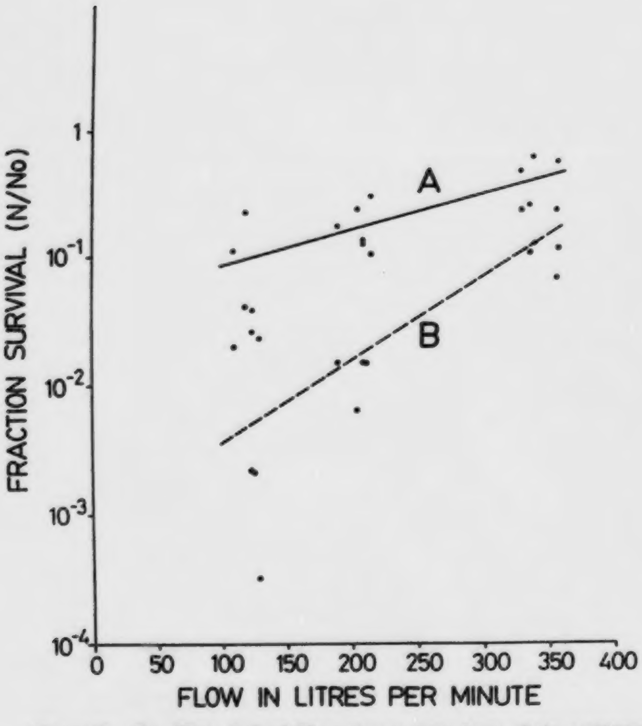

Figure 20: The effect of the 3.85 centimetre water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

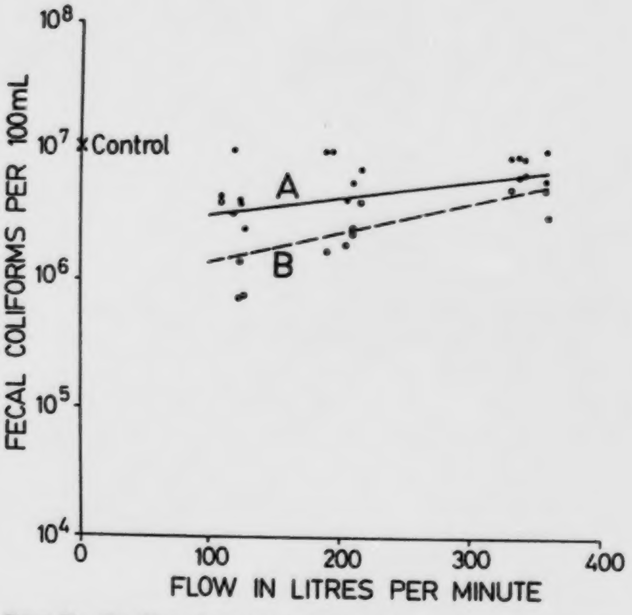

Figure 21: The effect of the 6.5 centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

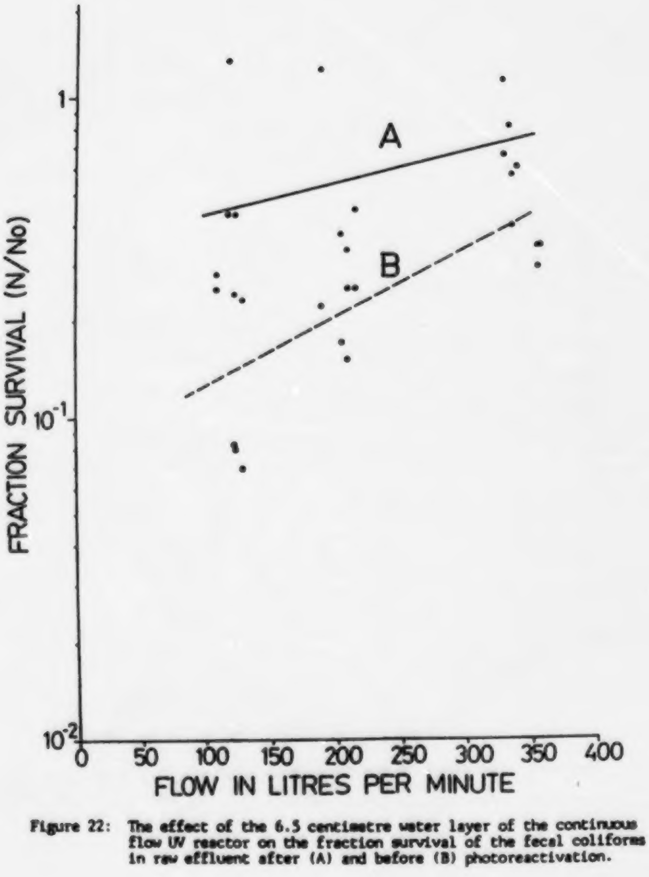

Figure 22: The effect of the 6.5 centimetre water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in raw effluent after (A) and before (B) photoreactivation.

The unfiltered raw effluent had a percent transmission of 12.7 (SD=1.7) at a wavelength of 254 nm. The level of suspended solids in the raw effluent was 179 mg/L (SD=41.2).

The dose of UV light in the continuous flow reactor can be calculated by dividing the germicidal wattage of the lamp by the flow rate in millilitres per second.

The total germicidal output of the 2000 W medium pressure lamp was 52.2 watts as measured in Phase 2. Ten percent of this output was subtracted for loses through the quartz sleeve. The dose of UV light in the continuous flow reactor at the slowest flow rate (110 L/min) was 26mW. sec/mL and 7mW. sec/mL at the highest flow rate (399 L/min). The experiments with the collimated beam showed that a dose of 13mW. sec/mL was required for a three logarithm reduction of the fecal coliforms without photoreactivation.

When the above doses of UV light are compared with those in the experiments with the collimated beam (Phase 2), the continuous flow reactor was unable to reduce the fecal coliforms to the same concentration or reach the same fraction survival.

The dry summer in London, Ontario, Canada had increased the suspended solids by 48 percent and decreased the transmission of the UV light by 54 percent. The increased concentration of suspended solids shields more fecal coliforms from the UV radiation and thus the limiting number of microorganisms which can be killed increases.

Mixing of the effluent is more complete during the experiments with the collimated beam and this will result in a greater kill of the fecal coliforms when the same volume of fluid is subjected to an identical dose of UV light.

A three logarithm kill of the fecal coliforms was obtained with flows of less than 172 litres per minute with the two centimeter water layer and 2000 watt medium pressure lamp. After photoreactivation, a three logarithm kill of the fecal coliforms was not obtained at any of the tested flow rates. Extrapolating the data beyond the tested flow rates may be invalid because the dose response curve is not linear over the entire range of UV doses. This is illustrated by the dose response curves in Phase 1 (Figures 1-8). If the response is linear, then the two centimeter water layer produces a three logarithm kill after photoreactivation at a flow rate of 14 litres per minute and the water layer with a depth of 3.85 cm produces a three logarithm kill without photoreactivation at a flow rate of 11 litres per minute.

As the kill of the fecal coliforms in the raw effluent increased there was a general increase in the degree of photoreactivation and this is in agreement with the research reviewed by Rupert (1964).

b. Primary Effluent

The level and fraction survival of the fecal coliforms in the primary effluent after irradiation with the 2000 watt medium pressure mercury lamp at various flow rates through three different water layers are shown in figures 23-28. Each Figure shows the level or fraction survival of the fecal coliforms before and after three hours in the sunlight.

The unfiltered primary effluent had a percent transmission of 27.1 (SD=1.7) at a wavelength of 254 nm. The level of suspended solids in the primary effluent was 45 mg/L (SD=6.7). The percent transmission was almost identical to that during the experiments with the collimated beam. The level of suspended solids was 27 percent less during the continuous flow studies.

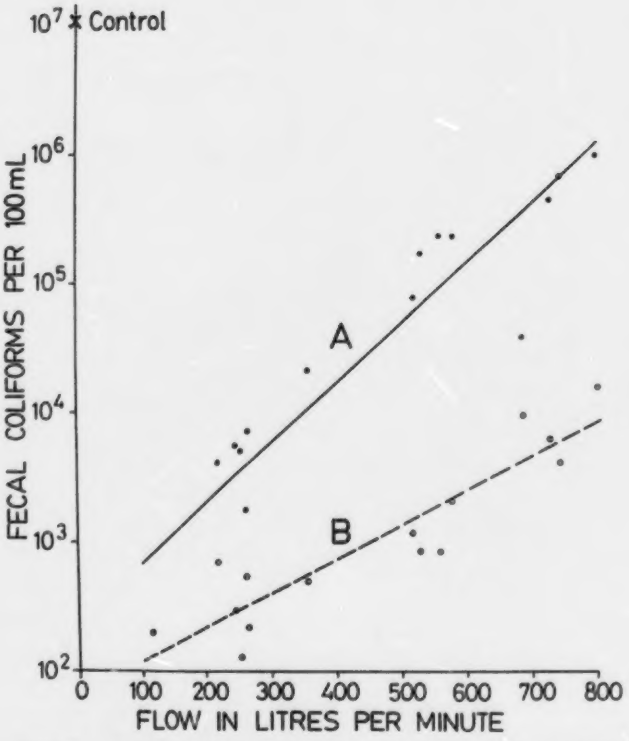

Figure 23: The effect of the two centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

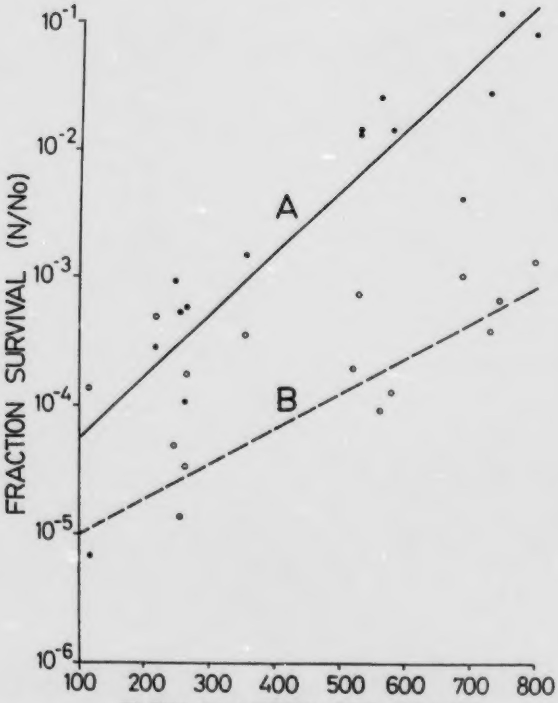

Figure 24: The effect of the two centimetres water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

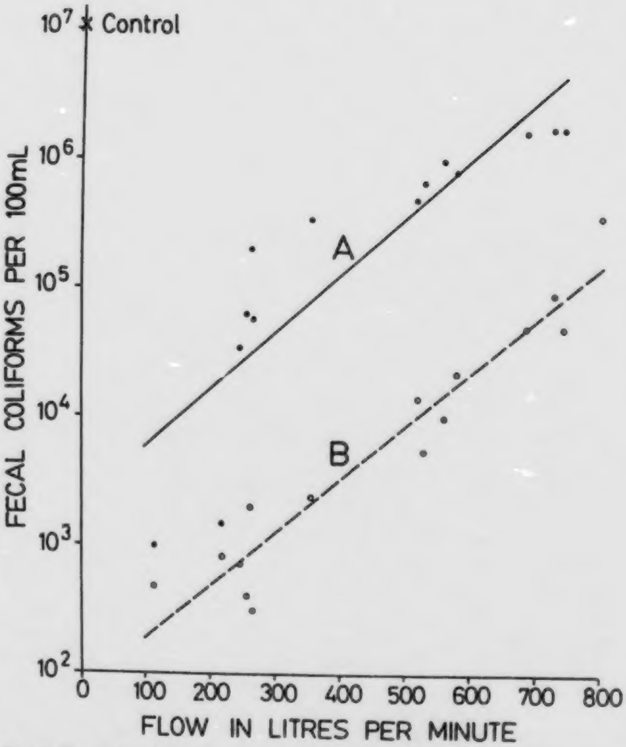

Figure 25: The effect of the 3.85 centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

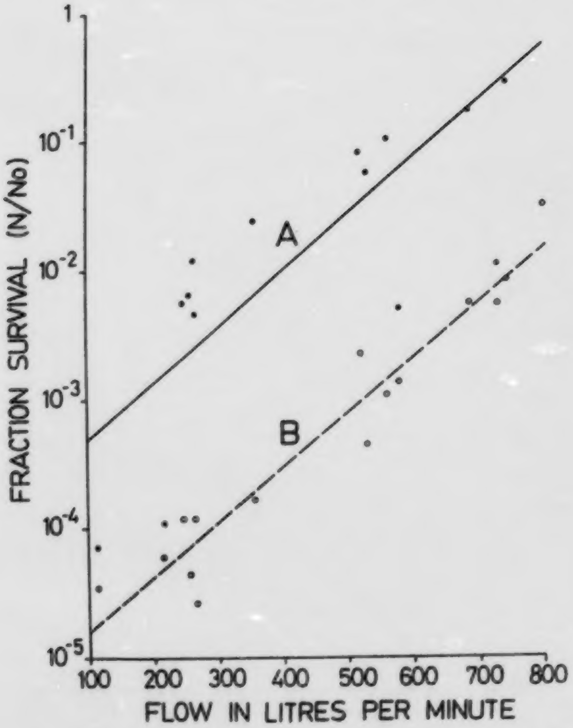

Figure 26: The effect of the 3.85 centimetre water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

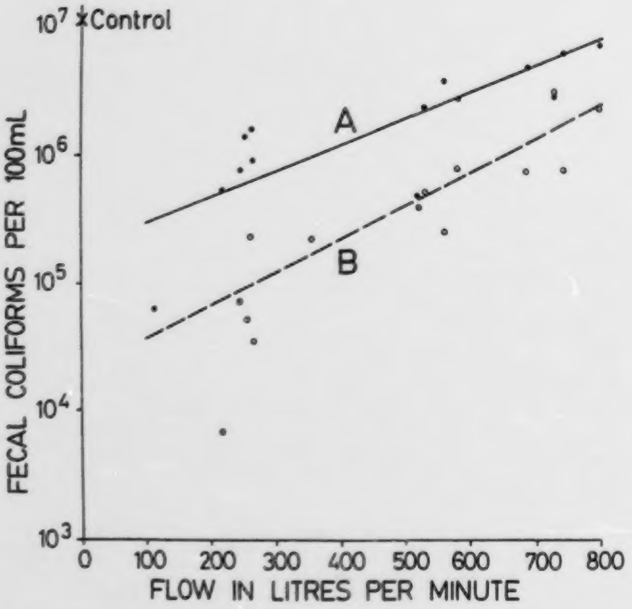

Figure 27: The effect of the 6.5 centimetre water layer of the continuous flow UV reactor on the number of fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

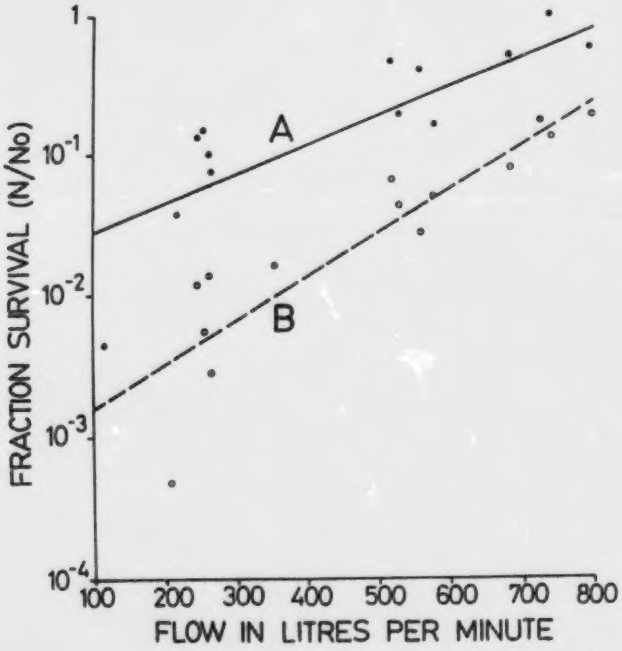

Figure 28: The effect of the 6.5 centimetre water layer of the continuous flow UV reactor on the fraction survival of the fecal coliforms in primary effluent after (A) and before (B) photoreactivation.

The dose of UV light in the continuous flow reactor was calculated in the same manner as it was for the raw effluent. The dose of UV light in the continuous flow reactor at the minimum flow rate (113 L/min) was 25 mW. sec/mL and 3.5 mW. sec/mL at the maximum flow rate (796 L/min). The experiments with the collimated beam in Phase 1 showed that a dose of 6 mW. sec/mL produced a three logarithm kill of the fecal coliforms without photoreactivation.

A comparison of the UV dose response between the continuous flow reactor and the experiments with the collimated beam showed that the two centimeter water layer attained the same or a better kill of the fecal coliforms at the high and low flow rates. Because the two sets of primary effluents were more closely related than the raw effluents, the mixing occurring during the flow of water through the two centimeter water layer must be similar to that occurring in the dishes used during the experiments with the collimated beam.

The 38.5 mm water layer also attained a three logarithm kill of the fecal coliforms before photoreactivation at the lowest flow rate which was tested. None of the combinations of flow rate and water layer produced a three logarithm kill of the fecal coliforms.

The data from the 65 mm water layer can be extrapolated to obtain the flow rate which produces a three logarithm kill of the fecal coliforms. This flow rate should be used with caution because the dose response curve is not linear over the entire UV dose range as shown in Figures 2-8. Without photoreactivation, the flow rate was 34 litres per minute for the 65 mm water layer.

Photoreactivation resulted in an average 1.3 logarithm increase in the fraction survival of the fecal coliforms in primary effluent. The average increase due to photoreactivation of the fecal coliforms in the raw and primary effluent after UV irradiation was one logarithm. This is similar to other studies with UV irradiated fecal coliforms in wastewater (Whitby et al. 1985 and 1984; Scheible and Bassell, 1981 and Bohm et al., 1982). There was no consistent increase or decrease in the degree of photoreactivation of the fecal coliforms in the primary effluent with a change in flow rate or water layer.

Conclusions

1. The two centimeter water layer with the 2000 W medium pressure mercury lamp approached the kill of the fecal coliforms obtained with the experiments with the collimated beam in Phase 1.

2. Mixing of effluents with high concentrations of suspended solids and low UV transmission is essential because each microbe must receive a minimum dose of UV light to be destroyed. Only the two centimeter water layer approached the proper mixing regime with raw and primary effluent.

3. A dose response curve of the fecal coliforms in low quality wastewaters should be prepared using static and continuous flow methods. The differences between the effluents and the results of the experiments with the collimated beam and the continuous flow reactor show the need for these types of experiments.

4. The UV equipment must be built for the worst conditions because a change in the quality of the effluent can dramatically effect the performance of the UV unit.

5. The continuous flow reactor was able to reach a three logarithm kill of the fecal coliforms with and without photoreactivation when treating primary effluent but only without photoreactivation when treating raw effluent.

COST ANALYSIS OF UV IRRADIATION FOR DISINFECTING LOW QUALITY WASTEWATERS

1. Introduction

An analysis of capital, operating and maintenance costs of disinfecting primary and raw effluents with medium pressure mercury lamps was undertaken to compare this disinfection alternative with chlorination and UV irradiation with low pressure mercury lamps.

2. Design Specification

a. Low Quality Wastewaters

Very little information is available which describes the UV transmission or concentration of suspended solids in low quality wastewaters. The simulated combined sewer overflow used by Zukovs et al. (1986) had a UV transmission at a wavelength of 254 nm of 2.8 percent per centimeter pathlength and the level of suspended solids was 187 mg/L. The primary effluent studied by Scheible et al. (1985) had a UV transmission at a wavelength of 254 nm of 55 percent per centimeter and the concentration of suspended solids was 80 mg/L. A review of the literature by Zukovs et al. (1986) showed that combined sewer overflow had levels of suspended solids between 80 and 274 mg/L.

Due to the above variations in low quality wastewaters, costs were developed for raw and primary effluent with the following UV transmissions at a wavelength of 254 nm and level of suspended solids. The raw effluent had a minimum UV transmission at a wavelength of 254 nm of 13 percent and a maximum concentration of suspended solids of 180 mg/L. The primary effluent had a minimum UV transmission at a wavelength of 254 nm of 27 percent and a maximum concentration of suspended solids of 45 mg/L.

b. Disinfection Standard

Phase 1 of this project showed that it was not practical to strive for a four logarithm kill of the fecal coliforms. Kollar et al. (1986) estimated their UV irradiation and chlorination costs with a four logarithm reduction of fecal coliforms but the results in this study show that this is not possible with UV irradiation.

A three logarithm kill of the fecal coliforms was obtained in the experiments with the collimated beam (Phase 1) and the continuous flow reactor (Phase 3). The UV equipment was costed to reach a three logarithm kill of the fecal coliforms.

If a three logarithm kill of the fecal coliforms after photoreactivation is required then it is not practical to treat raw effluent but it is possible to treat primary effluent. In raw effluent none of the combinations of flow rate and water layer reached a three logarithm kill of the fecal coliforms after photoreactivation. In primary effluent a three logarithm kill of the fecal coliforms was obtained after photoreactivation.

3. Disinfection Facilities

a. Introduction

UV equipment was designed for three different peak flow rates: 5,000, 50,000 and 500,000 m^3/day. The UV systems were designed for raw effluent without photoreactivation of the three logarithm kill of the fecal coliforms and for primary effluent with a three logarithm kill of the fecal coliforms before and after photoreactivation.

This cost analysis was for the capital, operating and maintenance cost for the UV equipment only and not the flow meters, buildings, cement work, etc.

b. Raw Effluent

The basic unit for estimating capital costs was a medium pressure mercury lamp and its associated hardware such a quartz sheath, ballast, starter, lamp supports, control box, cooling equipment and stainless steel shell. The capital costs for the UV equipment are shown in Table 7.

The operating and maintenance costs are shown in Table 7. The lamp replacement costs were estimated to be $300.00 per UV lamp. The UV lamps must be replaced every 5000 hours. Kollar _et al_. (1986) assumed that these facilities would operate only when required during the period from March to November, with total annual operating time assumed to be 250 hours. The lamp replacement frequency was assumed to be once every twenty years for the medium pressure mercury lamps.

After each use, the quartz sheaths should be acid washed to remove any deposits. A ten percent solution of phosphoric acid can be recirculated through the UV unit. This solution can be stored for further use. The estimated time required for cleaning was 4.5 to 8 hours. The capital cost for the inplace cleaning systems is shown in Table 8. The wage rate for a worker for the Ontario Ministry of the Environment is $13.41 per hour plus 25 percent benefits.

The power costs are shown in Table 7.

Table 7: Estimated costs for UV disinfection of raw effluent without photoreactivation of the fecal coliforms

	Flow Rate (m^3/d)		
Cost Component	5,000	50,000	500,000
Capital UV System	96,000	768,000	7,680,000
In Place Cleaning	14,000	22,000	150,000
Total Capital ($ 1988)	110,000	790,000	7,830,000
Operation and Maintenance			
Power (6¢/kwh)	2304	23,040	230,400
Labour ($16.76/h)	1676	2,095	3,352
Lamps	240	2,400	24,000
Chemicals	24	241	2,410
Total Operation and Maintenance ($/year 1988)	4,464	27,776	260,162

c. Primary Effluent

The two centimeter water layer was able to produce a three logarithm kill of the fecal coliforms with and without photoreactivation at flow rates of 360 and 825 litres per minute per UV lamp. The 3.85 centimeter water layer was able to produce a three logarithm kill fo the fecal coliforms without photoreactivation at a flow rate of 520 litres per minute per UV lamp. Extrapolation of the data with the 3.85 centimeter water layer showed that a three logarithm kill of the fecal coliforms after photoreactivation could be obtained at a flow rate of 175 litres per minute per UV lamp.

A flow rate of 370 and 147 litres per minute per UV lamp was used for estimating the number of lamps for a three logarithm kill of the fecal coliforms before and after photoreactivation, respectively. This is the average flow rate between the 2 and 3.85 centimeter water layer decreased by 45 percent for lamp aging.

The capital costs for the UV equipment are shown in Tables 8 and 9.

The operating and maintenance costs for primary effluent were estimated in an identical fashion to that of the raw effluent and are shown in Tables 8 and 9.

The annualized use-costs for UV disinfection of raw and primary effluent presented in Table 10 are based on a 20 year period and a discount rate of 7% as was used by Kollar _et_ _al_. (1986).

Table 8: Estimated costs for UV disinfection of primary effluent without photoreactivation of the fecal coliforms

	Flow Rate (m^3/d)		
Cost Component	5,000	50,000	500,000
Capital UV System	18,000	144,000	1,440,000
In Place Cleaning	7,000	11,000	75,000
Total Capital ($ 1988)	25,000	155,000	1,515,000
Operation and Maintenance			
Power (6¢/kWh)	432	4,320	43,200
Labour ($16.76/h)	1,676	1,676	2,095
Lamps	45	450	4,500
Chemicals	5	52	520
Total Operation and Maintenance ($/year 1988)	2,158	6,498	50,315

Table 9: Estimated costs for UV disinfection of primary effluent with photoreactivation of the fecal coliforms

	Flow Rate (m^3/d)		
Cost Component	5,000	50,000	500,000
Capital UV System	36,000	288,000	2,880,000
In Place Cleaning	14,000	22,000	150,000
Total Capital ($ 1988)	50,000	310,000	3,030,000
Operation and Maintenance			
Power (6¢/kwh)	864	8,640	86,400
Labour ($16.76/h)	1676	1,676	2,095
Lamps	90	900	9,000
Chemicals	9	86	860
Total Operation and Maintenance ($/year 1988)	2,639	11,302	98,355

Table 10: Estimated Use-Cost of Disinfecting Primary and Raw Effluents with Medium Pressure Mercury Lamps

Cost Component ($1988)	Flowrate (m^3/d) 5000	50,000	500,000
Raw, No Photoreactivation	2.97/m^3/d/yr	2.05	2.00
Primary, No Photoreactivation	0.90	0.42	0.39
Primary, Photoreactivation	1.47	0.81	0.77

The estimated use-costs of disinfection simulated combined sewer overflow and chemically treated primary effluent with low pressure mercury lamps are shown in Table 11. These costs were from the study of Kollar et al. (1986). To compare the two types of UV lamps only the capital and operating costs of the UV equipment itself were considered.

To compare UV disinfection with chlorination/dechlorination only the contact chamber, chemical storage tanks, pumps, piping, flow meter, injector system, evaporator, operation and maintenance were considered in the use-cost estimate from the work of Kollar et al. (1986) and these use-costs are shown in Table 12. The majority of the other capital costs are common to all of the forms of disinfection.

Disinfection of low quality wastewaters with UV light from medium pressure mercury lamps or with chlorination is lower than with low pressure mercury lamps.

Table 11: Estimated Use-Cost of Disinfecting Simulated Combined Sewer Overflow and Chemically Treated Primary Effluent with Low Pressure Mercury Lamps

Cost Component ($1985)	Flowrate (m^3/d) 5000	50,000	500,000
Simulated Combined Sewer Overflow	13.93/m^3/d/yr	12.94	12.04
Chemically Treated Primary Effluent	11.70	10.87	10.12

Table 12: Estimated Use-Cost of Disinfecting Simulated Combined Sewer Overflow and Chemically Treated Primary Effluent with Chlorination/Dechlorination and Chlorination, Respectively

Cost Component ($1985)	Flowrate (m^3/d) 5000	50,000	500,000
Simulated Combined Sewer Overflow	2.48/m^3/d/yr	1.36	1.04
Chemically Treated Primary Effluent	1.26	0.37	0.19

PROJECT CONCLUSIONS

1. Each type of wastewater required a different dose of UV light to reach the required level of disinfection due to the UV transmission, suspended solids and the relationship of the fecal coliforms with the solids.

2. To design a UV system for low quality wastewaters a series of survival curves should be prepared using the method with a collimated beam to determine whether the disinfection standard can be attained and the proper dose of UV light which is required to reach this disinfection standard. These results should be confirmed with continuous flow studies with a scale model of the projected UV equipment.

3. Medium pressure mercury lamps can reduce the fecal coliforms in raw and primary effluent by three logarithms in a static and continuous flow situation.

4. A use-cost comparison of UV disinfection of raw and primary effluent with medium pressure mercury lamps with that with low pressure mercury lamps showed that capital, operating and maintenance costs were lower with the former lamps.

5. Treatment of primary effluent by medium pressure mercury lamps without photoreactivation of the fecal coliforms was use-cost competitive with the chlorination of chemically treated primary wastewater. Chlorination and chlorination/dechlorination were lower in cost for the other effluents.

6. UV disinfection of low quality wastewaters may be an alternative to chlorination when the ecological considerations are taken into account such as the production of harmful chloro-organic compounds and residual of chlorine on the aquatic biota.

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B16

CHARACTERIZATION OF THE FECAL INDICATOR BACTERIAL FLORA OF SANITARY SEWAGE WITH APPLICATION TO IDENTIFYING THE PRESENCE OF SANITARY WASTE IN STORM SEWERS.

P.L. Seyfried, T. Bleier, Y. Xu and R. Harmandayan,
Department of Microbiology, FitzGerald Building,
University of Toronto, Toronto, Ontario

This study, sponsored by the Ministry of the Environment, investigated the use of specific bacteria to detect human fecal wastes in storm sewer lines. The organisms examined were fecal coliforms, *Escherichia coli*, fecal streptococci, enterococci, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Bifidobacterium* sp. These bacteria were isolated during periods of wet and dry weather from surface runoff, from designated locations in sanitary sewer lines, and from priority and non-priority storm sewers. Biochemical testing, serotyping, and/or genotyping were used to further characterize more than 4,000 fecal streptococcus, *Pseudomonas aeruginosa*, and *Bifidobacterium* isolates. Speciation of the fecal streptococci showed that *Streptococcus faecalis* subsp. *faecalis* was more predominant in sanitary and high priority sewers than in surface runoff and non-priority sewers. *S. casseliflavus*, on the other hand, was primarily found in runoff and non-priority storm sewers. DNA sequence studies of the fecal streptococci, using Restriction Endonuclease Analysis (REA) produced many different patterns and it was difficult to establish any relationship between the isolates. By comparison, *P. aeruginosa* genotypes were more uniform and fewer patterns were observed. As an example, a specific *P. aeruginosa* genotype was isolated from both the street runoff and the storm sewer at one City of Toronto location. Genotyping also appears to be a good method of distinguishing between the *Bifidobacterium sp.* that are predominant in sewage.

INTRODUCTION

The purpose of storm sewers is to collect storm water from urban areas and channel it into receiving waters such as rivers, streams, or lakes. Because storm sewer water does not receive any treatment prior to its discharge, it is important to identify any illegal sanitary connections in the storm sewer line. A small amount of dry weather flow would be expected in a normal or non-priority storm sewer; however, a priority storm sewer outfall that discharges more than 1 L/sec during dry weather and contains fecal coliform (FC) levels greater than 10,000 FC/100 mL is highly suspect and should be investigated. Because a method was needed to identify the source of human fecal pollution in storm sewers, a study characterizing the bacterial indicators found in both sanitary and storm sewers was initiated in the fall of 1986.

In the first year of the study, samples were collected from surface runoff when it rained and from specific locations in sanitary sewer lines and in priority and non-priority storm sewers during periods of wet and dry weather. Fecal coliforms, *Escherichia coli*, fecal streptococci, enterococci, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *Bifidobacterium* sp. were enumerated in each of the samples. The results, presented in Part D of the 1987 Technology Transfer Conference Proceedings (Seyfried *et al.*, 1987) showed that higher densities of all indicator organisms, including *Bifidobacterium*, were recovered from priority storm sewer samples than from non-priority sewage samples. It was found that changes in bacterial indicator populations were best observed during periods of dry weather.

The objective of the segment of the study described herein was to analyze more dry weather samples and further characterize, by means of biochemical testing, serotyping and/or genotyping, the fecal streptococci, *Pseudomonas aeruginosa*, and *Bifidobacterium* isolates from sanitary and storm sewers.

METHODS

Sampling Sites

Sites A, B and C (shown in Table 1 and Fig. 1) in the Mount Steven Trunk storm sewer line were sampled because this area was designated a high priority sewer by the Ministry of the Environment. The non-priority sites, selected for comparison, were X, Y and Z in the Mount Steven Trunk storm sewer branch lines. During periods of wet weather, storm water run-off was also collected at the X, Y and Z sites. These samples were labelled R, G and Q, respectively. Samples D, E and F were obtained from a sanitary sewer in close proximity to the priority storm sewer sampling points.

Sample Collection

During periods of dry weather, triplicate samples were collected from each sampling point in the sewers over a four-day period. The dry weather samples were obtained in October, 1986; June, 1987; August, 1987; June, 1988; and August, 1988. Wet

TABLE 1. Sampling locations of high priority and non-priority storm sewers, sanitary sewer and storm water runoff.

Sample Description	Code	Site
High Priority Storm Sewer Line, Mount Steven Storm Sewer Trunk	A	Danforth and Jones Avenues - furthest in-line sampling point (near source of suspected solution input).
	B	Pape and Strathcona Avenues (mid-line sampling point).
	C	First and Broadview Avenues (near outfall).
Non-Priority Storm Sewer Branch Lines	Y	Chatham and Jones Avenues (connects to main line above sampling point B).
	X	Danforth And Woodycrest Avenues (connects to main line above sampling point A).
	Z	Pape and Cavell Avenues.
Storm Water Runoff	G	Chatham and Jones
	R	Danforth and Woodycrest Avenues
	Q	Pape and Cavell Avenues
Sanitary Sewage Line	D	Danforth and Jones Avenues
	E	Strathcona and Pape Avenues
	F	First and Broadview Avenues

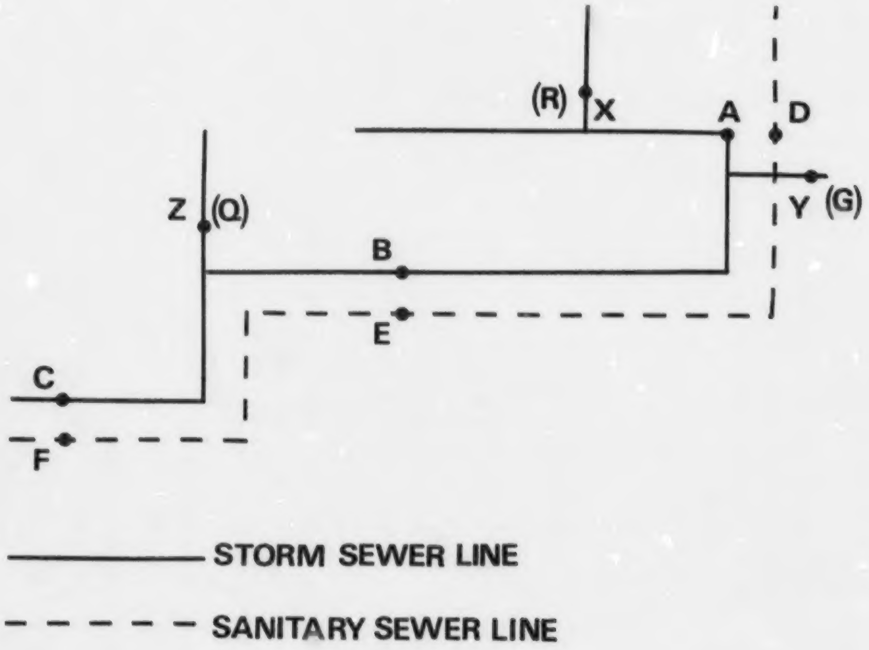
(R)
X
A
D
Z
(Q)
Y
(G)
B
E
C
F
STORM SEWER LINE
SANITARY SEWER LINE

FIG. 1 - Schematic diagram of Mount Steven storm sewer and sanitary sewer lines.

weather samples were collected from the sewers and the street run-off during rainy days in July, September and October, 1987.

The samples were collected in sterile glass containers, transported to the laboratory on ice, and processed within 6 hours of collection.

Fecal specimens from both humans and animals were also obtained.

Bacterial Isolation and Enumeration

Samples were analyzed for fecal coliforms using m-TEC agar (Dufour, 1981), *Escherichia coli* by means of the m-TEC urease treatment described by Dufour (1975; 1981), fecal streptococci isolated on m-Enterococcus agar (Slanetz and Bartley, 1977), enterococci on m-ME agar (Dufour, 1980), *Pseudomonas aeruginosa* using m-PA agar (Standard Methods, 1985) and *Bifidobacterium* sp. isolated on the YN-17 medium described by Mara and Oragui (1983).

All bacteria were identified to the species level using standard taxonomic methods (Seyfried *et al.*, 1987).

Bacterial Characterization

Approximately 2,500 fecal streptococci were recovered on m-Enterococcus agar and m-ME agar during the dry and wet weather surveys. The isolates that were identified as varieties of *S. faecalis* were tested for their reaction in litmus milk broth (Difco) using the method of Mundt (1973).

Serotyping of the *P. aeruginosa* isolates was carried out using a Pseudomonas Antisera Kit (Difco). The organisms were subspeciated into the 17 different heat-stable somatic antigen groups described by Kusama (1978).

For the restriction enzyme analysis (REA) of P. aeruginosa, total cellular DNA was extracted using a method described by Bradbury et al. (1984, 1985). A 1.5 mL volume of an 18 hour nutrient broth culture inoculated with P. aeruginosa was transferred into an Eppendorf tube and centrifuged in a Microfuge 12 (Beckman) for 3 minutes at 7500 x g. The supernatant was discarded and the pellet loosened by vortexing. A 291 µL volume of PEB I buffer containing 10 mg/mL lysozyme was added and the mixture incubated for 20 minutes at 35° C. A 9 µL amount of 5M NaCl was added, thoroughly mixed, and then 150 µL of 10% SDS was added. The solution was gently mixed and incubated for 10 minutes at 37° C. Following the addition of 450 µL phenol:chloroform:isoamyl (25:24:1), the mixture was vortexed and centrifuged at 7500 x g for six minutes at room temperature. The upper aqueous phase was removed with a pasteur pipette and transferred to an Eppendorf tube. One mL of 95% cold ethanol was added, the tubes vigorously shaken and stored at -20° C overnight. The mixture was centrifuged for 3 minutes at 12,000 x g, the supernatant discarded and the pellet redissolved in 250 mL DNA wash buffer. A total of 500 µL of 95% cold ethanol was added, the mixture stored at -20° C for 20 minutes, and centrifuged at 1,200 x g for 3 minutes. The supernatant was discarded and the pellet allowed to dry at 37° C for 10 minutes. The pellet was dissolved in 100 µL of distilled water and stored at 4° C until digested.

Restriction digests were performed using Sma I according to the manufacturer's instructions (Boehringer Mannheim). A 10 µL aliquot of double strength (2X) Sma I buffer was placed in an Eppendorf tube and 10 uL extracted DNA added. A 2 µL sample of Sma I enzyme was added and the mixture was incubated for 1 hour at 37° C to allow for complete digestion. Following the addition of 1 µL of 0.15M EDTA + 0.4 mg/mL RNase A, the tube was incubated at 37° C for 20 minutes. A 5 µL volume of 5X sample buffer was then added to the restriction digest. Samples were electrophoresed on 0.7% agarose gel for 16 hours at 27 volts. Gels were stained with 1 mg/mL ethidium bromide in 1X TAE (Tris base, 1.0 sodium acetate, 0.1 M disodium EDTA) for 1 hour and destained for 2 hours in distilled water. Photography was done using U.V. light at 300 nm and a red No. 23A polaroid 665p/N film with an exposure time of 30 seconds.

Fecal streptococci were grown in Brain Heart Infusion broth (Difco) at 37° C for 4 hours and the total cellular DNA extracted as described previously. Restriction digests were performed using Bam HI enzyme according to the manufacturer's directions (Boehringer Mannheim) along with a 10 µL aliquot of 2X Bam HI buffer.

Bifidobacterium sp. were grown anaerobically in MRS broth (Oxoid) for 48 hours at 37° C. Three different enzymes, Sma I, Bam HI and Cfo, with their appropriate buffers, were used for the restriction enzyme analysis of the bifidobacteria.

RESULTS AND DISCUSSION

Data from the previous study (Seyfried et al., 1987) suggested that fecal contamination, possibly human in origin, was evident in the storm sewer line near sites A and Y. The results of the 1988 survey, presented in Table 2, add support to this conclusion. As may be seen in the table, the fecal coliform levels in the high-priority storm sewer were greater than 10,000/100 mL at site A in June and at all sites in August. It should be noted that counts of all indicator organisms tended to be higher in August, possibly due to regrowth of the bacteria in the warmer nutrient-enriched waters (Hoadley, 1977).

As might be expected, fecal coliform and fecal streptococcus counts were highest in sanitary sewage and the fecal coliform to fecal streptococcus ratio was greater than 4 in these samples. Although a ratio greater than 4 was observed at site A in the priority storm sewer, at other storm sewer sites the FC/FS ratios were generally below 4 indicating that there was little or no human fecal input (Geldriech and Kenner, 1969).

P. aeruginosa and Bifidobacterium sp. were shown in the 1987 study to have potential as indicators of human fecal waste. Collaborative data presented in Table 2 shows that counts of both organisms were higher in sanitary and priority storm sewage than in non-priority storm sewage.

Mara and Oragui first proposed the use of sorbitol fermenting bifidobacteria as indicators of human fecal pollution

TABLE 2. Overall geometric mean concentrations of fecal coliforms, *E. coli*, Fecal Streptococci, Enterococci, *P. aeruginosa*, and *Bifidobacterium* sp. recovered from sanitary sewage as well as high-priority and non-priority storm sewage during two dry weather surveys in 1988.

Survey	Site	Fecal Coliforms	*E. coli*	Fecal Streptococci	Enterococci	Pseudomonas aeruginosa	Bifidobacterium
Dry Weather June, 1988	Sanitary Sewage						
	D	9.77×10^5	6.91×10^5	1.74×10^5	1.45×10^5	2.69×10^4	NA
	E	1.00×10^6	8.51×10^5	1.90×10^3	2.75×10^5	7.08×10^3	NA
	F	3.31×10^4	3.31×10^4	1.51×10^4	6.46×10^3	1.00×10^4	1.58×10^4
	High Priority Storm Sewage						
	A	1.57×10^4	5.94×10^3	6.80×10^3	1.31×10^3	2.62×10^2	NA
	B	5.88×10^3	3.72×10^3	9.35×10^3	4.33×10^3	1.90×10^2	NA
	C	5.62×10^3	3.09×10^3	7.43×10^4	5.77×10^4	2.75×10^2	NA
	Y	7.76×10^2	5.57×10^2	2.54×10^3	1.01×10^3	3.11×10^1	NA
	Non-priority Storm Sewage						
	X	9.77×10^2	7.94×10^2	4.57×10^4	3.16×10^4	1.78×10^1	NA
	Z	2.29×10^2	1.48×10^2	8.13×10^3	2.75×10^2	1.29×10^1	7.59×10^3
Dry Weather August, 1988	Sanitary Sewage						
	D	1.53×10^8	1.35×10^8	2.64×10^6	1.26×10^6	2.68×10^4	NA
	E	1.05×10^7	6.30×10^6	2.26×10^4	6.85×10^3	2.82×10^6	NA
	F	1.42×10^4	9.56×10^3	2.43×10^3	1.76×10^3	3.65×10^1	5.8×10^4
	High Priority Storm Sewage						
	A	1.21×10^5	1.65×10^4	4.46×10^4	4.07×10^4	1.14×10^3	NA
	B	1.00×10^4	5.25×10^3	2.00×10^3	2.57×10^2	NA	NA
	C	2.64×10^4	1.48×10^4	7.59×10^4	3.83×10^4	1.18×10^1	1.26×10^5
	Y	5.41×10^4	2.13×10^4	5.27×10^4	5.25×10^4	1.26×10^4	2.29×10^4
	Non-priority Storm Sewage						
	X	4.07×10^5	3.63×10^4	3.03×10^3	3.92×10^2	2.88×10^3	NA
	Z	2.88×10^4	1.02×10^4	3.24×10^2	2.34×10^2	3.80×10^2	NA

in 1983. Kator and Rhodes (1988) also used these organisms to differentiate human from animal sources of pollution in shellfish growing waters. The species of *Bifidobacterium* that are reportedly human specific and sorbitol fermenting are *B. adolescentis* and *B. breve*. In this study we were able to isolate *B. breve* and *B. bifidum* from human fecal material; however, we also recovered *B. adolescentis* from dog fecal samples and *B. breve*, *B. minimum* and *B. thermophilum* from chicken feces. Twenty-one isolates that were thought to be *Bifidobacterium* on the basis of their morphology were recovered from eight different sewage samples. Of the 21 isolates, only two could be identified by biochemical testing. The two were found in the non-priority storm sewer at site Z and were classified as mannose + and mannose - strains of *B. thermophilum*.

Based upon our prior use of restriction enzyme analysis to distinguish between different strains of *Klebsiella pneumoniae* (Seyfried *et al.*, 1989), it was felt that genotyping might assist in determining the source of the *Bifidobacterium* strains under investigation. Four different enzymes were used to digest whole cell DNA from *B. adolescentis* and two *Bifidobacterium* sp. isolated form chicken feces. The restriction (REA) patterns demonstrated by the total cellular DNA restriction enzyme analysis are shown in Fig. 2. As might be expected, the chicken fecal isolates appeared to have very similar patterns. Bam HI seems to be the enzyme of choice for the digestion of *Bifidobacterium*, and it will be used in all future genotyping

Figure 2. Agarose (0.7%) gel electrophoresis of total cellular DNA from *B. adolescentis* and *Bifidobacterium* fecal isolates.

Lanes 1 to 3 contain whole cell DNA from *B. adolescentis* digested with Sma I, Bam HI and Cfo, respectively.

Lanes 4 to 6 contain whole cell DNA from a chicken fecal isolate of *Bifidobacterium* digested with Sma I, Bam HI, and Cfo, respectively.

Lanes 7 to 9 contain whole cell DNA from a *Bifidobacterium* isolated from chicken feces digested with Sma I, Bam HI, and Cfo, respectively.

experiments.

Serotyping of the P. aeruginosa isolates showed that serotype 6 predominated in the priority and non-priority storm sewage, sanitary sewage and storm water runoff. Serotypes 1, 11, 4, 3 and 2, although not as prevalent as 6, were also common in all categories of samples.

Seventy-eight strains of P. aeruginosa from the sanitary sewer and 113 strains recovered from the storm sewer and storm water runoff were also genotyped. Forty-six different REA patterns were noted among the 191 isolates. As may be seen from Table 3, there was an interesting distribution of patterns among the sample groups. For example, REA patterns 1', 6, 6' and 13' were found among isolates from the three sampling sites in the sanitary sewer. The fact that these same patterns or genotypes were also prevalent in the priority storm sewer samples suggests that they may be typical of human fecal isolates. A comparison of the corresponding serotypes for each genotype showed that the serotypes tended to be widely distributed. For example, the 1' genotype was found in serotypes 1, 6 and 10; REA pattern 6 was distributed among serotypes 1, 3, 4, 6 and 11; 6' occurred in serotypes 1, 6, 9, 10 and 11; and the 13' genotype was found in serotypes 6 and 10. Because it seemed unusual to find such a high number of genotype 6 isolates in the non-priority storm and runoff samples, the source of these organisms was examined. It was found that the bacteria were all isolated during the wet weather sampling in July, 1987 from sites X and P. The P site was

TABLE 3. Distribution of the prominent Pseudomonas aeruginosa REA patterns among sanitary sewer, priority and nonpriority storm sewer and storm water runoff samples.

REA Pattern 46[a]	Sanitary Sewer Sites D,E,F 78	Priority Storm Sewer Sites A,B,C,Y 49	Nonpriority Storm Sewer Sites X,Z 29	Storm Water Runoff Sites P,Q,G,R 35
1'	8 (10.2)*	7 (14.3)	0 (0.0)	2 (5.7)
6	6 (7.7)*	4 (8.2)*	5 (17.2)	10 (28.6)
6'	4 (5.1)	3 (6.1)	0 (0.0)	1 (2.80)
13'	8 (10.2)*	5 (10.2)	0 (0.0)	0 (0.0)
18	0 (0.0)	0 (0.0)	4 (13.8)*	6 (17.1)
20	0 (0.0)	0 (0.0)	2 (6.9)	3 (8.6)

[a] Total number of REA patterns or samples in each category.

* Found at all sites in the sample category (e.g. sanitary sewer, sites D, E and F).

() Percentage

an additional street runoff sample taken at the beginning of the rainfall event. These isolates, belonging to REA pattern 6, were evenly divided between serotypes 1 and 6.

Additional information not provided in Table 3 is that genotypes 1, 2, 4, 7, 9, 11′, 12 and 14 were all isolated from sanitary and priority storm sewers and not from non-priority storm sewers or storm water runoff.

In comparison, P. aeruginosa isolates with genotypes 18 and 20 were found solely in the non-priority sewer and storm runoff samples. The REA pattern 18 organisms were distributed between serotypes 3 and 6, whereas pattern 20 was found in serotypes 1 and 3. Serotype 3, REA pattern 18 P. aeruginosa isolates were found in the storm water runoff samples at site Q, and in the sewer that collected this same storm water at site Z. Similarly, REA pattern 20 isolates were recovered from water runoff at site R and in the receiving storm sewer at site X. The fact that the genotype 20 organisms belonged to both serotypes 1 and 3 suggests that genotyping is probably a more concise method of fingerprinting P. aeruginosa than serotyping. From the results, genotyping appears to be a promising method of tracing P. aeruginosa from human and animal sources.

Compared with P. aeruginosa, genotyping of the fecal streptococci did not produce any concise results. One hundred and ninety-two streptococcal isolates, 60 of which were from the sanitary sewer, were genotyped. A total of 64 different REA patterns were identified among the isolates.

Streptococcus faecalis subsp. *faecalis* was the only species that had the predominant genotypes 5 and 8 occurring in both the sanitary sewer and the priority storm sewer isolates. Mundt (1973) has suggested that *S. faecalis* isolates from human feces will produce an acid curd in litmus milk. However, no relationship between the 5 and 8 pattern isolates and those that produced an acid curd was observed. Although the *S. faecium* isolates were distributed among 31 different REA patterns, genotype 9 was found in all three sites of the sanitary sewer and genotype 5 was recovered from all sites in the priority storm sewer. *S. faecium* subsp. *casseliflavus* had 21 different REA patterns that were widely distributed among the isolates from different sources. In general, no relationship between the source of isolation and the genotype could be found among the 192 streptococcal isolates studied.

More insight into the origin of fecal wastes in storm sewer lines was provided by the speciation of fecal streptococci. The results showed that *S. faecium* tended to be equally represented in all sample categories. On the other hand, *S. faecalis* subsp. *faecalis* (Fig. 3) was found more frequently in sanitary and priority storm sewers than in surface runoff and non-priority sewers. In contrast, *S. faecium* subsp. *casseliflavus* (Fig. 4) predominated in non-priority storm sewer water and was notably evident in storm water runoff. The organism was virtually nonexistent in sanitary sewage and levels in priority storm sewers were small. These results concur with our previous data

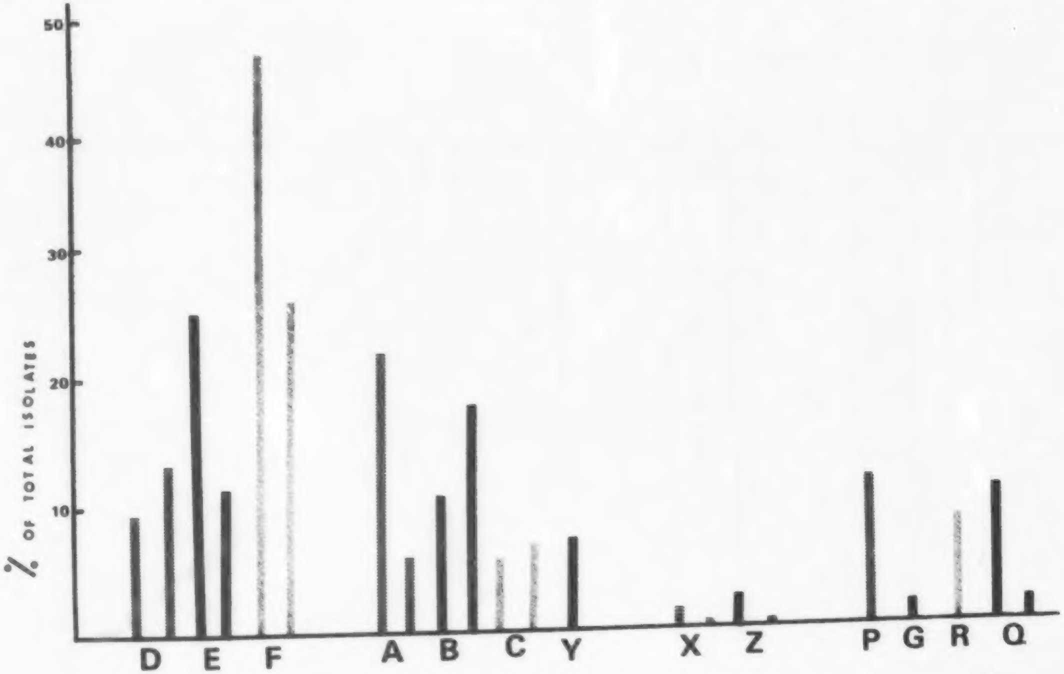

Fig. 3 Percentage distribution of Streptococcus faecalis subsp. faecalis among the sanitary sewage sites (D,E,F), the priority storm sewer (A,B,C,Y), the non-priority storm sewer (X,Z) and the storm water runoff (P,G,R,Q) locations.

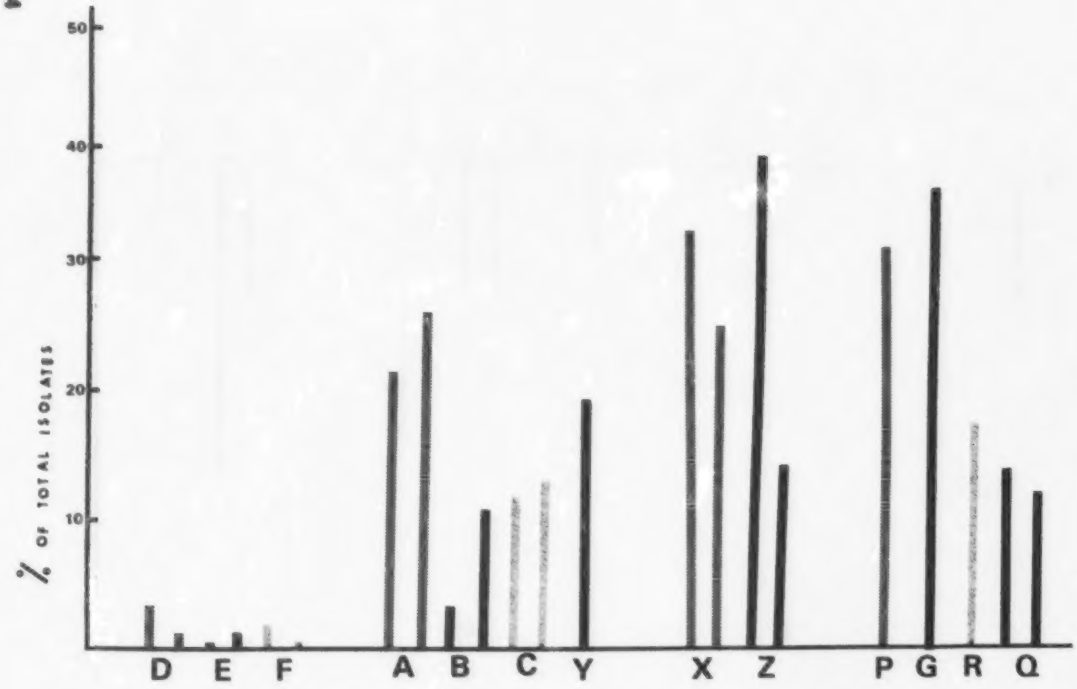

Fig. 4. Percentage distribution of Streptococcus faecium subsp. casseliflavus among the sanitary sewage sites (D,E,F), the priority storm sewer (A,B,C,Y), the non-priority storm sewer (X,Z), and the storm water runoff (P,G,R,Q) locations.

(Seyfried, Harris, Young, 1986 unpublished) which showed that S. faecium subsp. casseliflavus could be isolated from animal feces but not from human fecal specimens.

Conclusions

The conclusions that may be drawn from this segment of the study are as follows.

1. The levels of fecal coliforms, Escherichia coli, P. aeruginosa and Bifidobacterium sp. suggest that there is an impact near site A in the storm sewer line that may be due to human fecal pollution.
2. While Bifidobacterium breve and B. adolescentis are found in human feces, they cannot be used to differentiate human from animal sources of pollution because they can be isolated from animals such as dogs and chickens.
3. Genotyping of Bifidobacterium isolates may provide a more precise method of source differentiation.
4. P. aeruginosa genotyping may be of value in tracing sources of pollution. Serotyping, however, does not provide specific results.
5. Genotyping of fecal streptococci is not recommended as a method of source determination since the wide variety of patterns produced yield inconclusive results.
6. Speciating fecal streptococci is a useful means of characterizing sewer or storm water content. For example, S. faecalis subsp. faecalis is found predominantly in sanitary

and priority storm sewers whereas *S. faecium* subsp. *casseliflavus* is characteristically present in non-priority storm sewers and storm water runoff. To date, *S. faecium* subsp. *casseliflavus* has not been isolated from human feces.

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LANDSAT-5 SPECTRAL RESPONSES FOR LAKES ACROSS NORTHEASTERN ONTARIO

J. Roger Pitblado and Dale A. Dempsey

Department of Geography
Laurentian University
Sudbury, Ontario P3E 2C6

INTRODUCTION

Digital data from the Multispectral Scanner (MSS) of the Landsat series of satellites have been employed since the launch of Landsat-1 in 1972 for a wide range of lake water quality assessment programs (Brooks, 1975; Fisher et al., 1979; Scarpace et al., 1979; Lillesand et al., 1983; Verdin, 1985). These activities have only been partially successful due to the relatively coarse spatial, spectral, and radiometric resolution of MSS data (Middleton and Munday, Jr., 1980; Witzig and Whitehurst, 1981; Hilton, 1984; Pitblado, 1984; Lindell, 1986). But with the launch of Landsats 4 and 5 in 1982 and 1984, respectively, satellite water quality assessments have been enhanced because of the following significant design improvements of the Thematic Mapper scanner (after Lathrop, Jr. and Lillesand, 1986):

- 30-m versus 80-m ground resolution in the visible and reflected infrared bands;
- seven bands of sensing versus four bands, notably with a new band in the blue wavelength region and a thermal infrared band;
- 8-bit versus 6-bit radiometric resolution, with the consequent 256-level recording of data compared to 64-level.

In the latest issue of Photogrammetric Engineering and Remote Sensing, another satellite monitoring tool for water quality assessments is reported on - the French SPOT satellite, launched on 21 February 1986. Merry et al. (1988) discuss the use of the 20-m multispectral mode of SPOT HRV data for mapping relative ranges of suspended sediment concentrations in Lake Erie. Unfortunately, despite the increase in spatial resolution, SPOT data will have limited appeal to water managers and scientists because of the lack of a band in the blue portion of the spectrum.

Studies in Northeastern Ontario using satellite imagery for water quality assessments have focussed on the use of Landsat digital data, with most success coming from the use of TM as opposed to MSS scanner data. In feasibility studies designed to discriminate between clear acidic lakes from non-acidic lakes, classifications in the order of 90% correct have been achieved (Pitblado et al., 1987; Pitblado, 1987a 1987b and 1988).

Expanding beyond the feasibility study of 1986-87, the authors are now engaged in a three-year research project funded by the Ontario Ministry of the Environment (RAC Project No. 354G). The general aim is to characterize and map the lakes in three selected areas of Northern Ontario using Landsat TM data. This paper describes some of the work undertaken in the first eight months of that study. The objective today is to describe the nature of the TM data that have been acquired for Northeastern Ontario and identify a limited number of associations between those data and a selected water quality parameter. More specifically, use is made of the techniques of principal components analyses (PCA) and discriminant analyses of TM data (from Landsat-5) in association with measured values of dissolved organic carbon (DOC).

MATERIALS AND METHODS

Study Area and Landsat Imagery

Spectral responses acquired by the Landsat-5 Thematic Mapper scanner (TM) are being gathered for all water bodies larger than one hectare (if they are detectable by the satellite) in the area of Northeastern Ontario that extends from the North Channel of Lake Huron to Highway 11 (south-north) and from Wawa to Temagami (west-east), an area of approximately 120,000 sq.km. The region is roughly outlined by the location of 633 of the study lakes in Figure 1. Given the scale of the map and the small size of many of the lakes, some of the dots there may represent the location of up to two of the lakes sampled for analyses.

For the purposes of discussion here, the mean values of the TM responses for all of the seven TM channels for each of 633 lakes have been employed. The TM channels include: the visible bands TM1 (450-520 nm), TM2 (520-600 nm), TM3 (630-690 nm); the near- to mid-infrared bands TM4 (760-900), TM5 (1550-1750), TM7 (2080-2350 nm); and a thermal channel TM6 (10400-12500 nm). Because a relatively slow and size-limited microcomputer (the IMAVISION system of PCI Inc. of Richmond Hill, Ontario) was employed to acquire these spectral statistics the authors designed a sampling program and computer procedures that would remove atmospheric haze (Richards, 1986), eliminate non-water pixels, perform a principal components analysis on the "water" pixels, outline the water bodies with a polygon-border, and then compute and record the descriptive statistics for each of the sampled water bodies. As the study area is very large, twenty-one TM quadrants were used from the Landsat-5 images listed in Table 1. The routine described above was applied to a quadrant from one to five times (depending on the location and distribution of candidate lakes) with each application requiring approximately two to three hours of automated and interactive processing time.

Figure 1. The study area is outlined by the location of the lakes in this sketch map of the northern portion of the Province of Ontario. Note the lack of study lakes in the Timmins area where no cloud-free summer imagery was available.

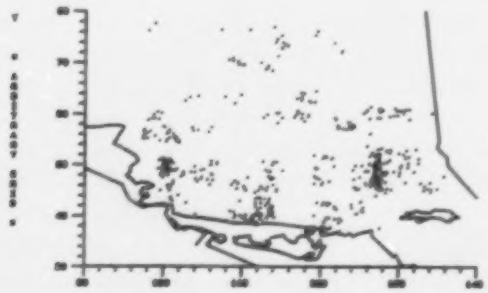

Table 1. Listing of the Landsat-5 Thematic Mapper Images Used in the Analysis of Lake Spectral Responses in Northeastern Ontario. No single year could be employed because of varying cloud cover but the images selected were restricted to July and August - to match the lake water quality sampling dates.

Path/Row	Date of Image	Scene I.D.	
19/27	13-8-86	50895-153030	Quad. 1
19/27	13-8-86	50895-153030	Quad. 3
19/27	31-7-87	51247-153429	Quad. 12
19/28	13-8-86	50895-153047	Quad. 1
20/26	17-8-85	50534-154406	Quad. 1
20/27	1-8-85	50518-154445	Full Scene
20/28	1-8-85	50518-154503	Quad. 1
20/28	1-8-85	50518-154503	Quad. 2
21/26	7-7-85	50493-155044	Full Scene
21/27	26-7-86	50877-154331	Full Scene
21/27	7-7-85	50493-155101	Quad. 2
22/27	18-8-86	50900-154851	Quad. 2
22/27	18-8-86	50900-154851	Quad. 4

Water Parameter Data

Water parameter data for the sampled lakes were compiled from a number of sources but the objective was to obtain these data from lakes that were sampled within a July-August window as close as possible to a Landsat overpass.

This objective was met for a large number of lakes in the Sudbury and Algoma areas because of the field work undertaken by the senior author in 1986 (Pitblado, 1987a). For those lakes, which make up close to 50% of the study lakes, the difference in timing between Landsat data acquisition and field data acquisition ranged from a few minutes to one week. However, the remaining data acquisitions depended on accessing the databases of the Ministries of Environment and Natural Resources (see Acknowledgements) and matching with cloud-free Landsat imagery. Consequently the timing differences for some of the remaining study lakes could be as large as one full year.

In effect, four lake databases (noting that the four do have significant overlap due to the sharing of data between Ministries; also, for analytical methods see MOE, 1981) were employed: Pitblado/MOE, 1987a; the MOE A.P.I.O.S. datasets described by Pitblado and Keller (1984) and Keller and Pitblado (1986); the Inland Lake Database from MOE Dorset; and the Aquatic Habitat Inventory of the Ontario Ministry of Natural Resources. The water parameters (and some of their descriptive statistics) that were acquired from these databases are listed in Table 2. That table highlights some of the unevenness in parameter acquisition and, in the opinion of the authors, is a further argument for the use of remote sensing to gather data for selected water parameters in regions where lakes are numerous, remote in location, and scattered over an enormous geographical area.

Table 2. Descriptive Statistics for the Lake Water Parameters Compiled for this Study.

Minimum	Maximum	Mean	(n)	Parameter
.100	10.500	2.931	201	DOC (mg/L)
2.200	21578.900	825.225	540	Lake area (ha)
.500	39.700	8.226	472	Lake depth (m)
.100	4.900	1.472	134	Chlorophyll a (µg/L)
.500	22.000	5.586	330	Secchi depth (m)
-1.800	106.700	14.058	156	Apparent colour (hazen units)
4.300	8.200	6.263	369	pH
-2.740	116.640	7.242	369	TIP Alkalinity (mg/L)
.000	39.120	5.106	244	TFE Alkalinity (mg/L)
12.000	400.000	49.285	403	Conductivity (µmhos/cm)
40.000	660.000	192.331	251	TKN (µg/L)
.000	124.000	25.979	143	NH3 (µg/L)
1.000	86.000	1.965	143	NO2 (µg/L)
5.000	265.000	41.755	143	NO3 (µg/L)
1.000	30.000	5.988	251	Total P (µg/L)
.720	33.900	3.752	281	CA (mg/L)
.210	7.100	.950	281	MG (mg/L)
.250	22.300	.904	254	NA (mg/L)
.100	1.700	.424	254	K (mg/L)
2.770	29.200	9.347	281	SO4 (mg/L)
.030	2.530	1.051	143	SiO3 (mg/L)
.010	40.700	.775	251	CL (mg/L)
6.000	760.000	106.525	276	AL (µg/L)
1.000	290.000	50.430	272	MN (µg/L)
5.000	1275.000	62.257	253	FE (µg/L)
1.000	41.000	6.956	251	ZN (µg/L)
1.000	46.000	2.833	251	CU (µg/L)
1.000	290.000	9.135	251	NI (µg/L)
3.000	13.000	3.343	143	PB (µg/L)

RESULTS AND DISCUSSION

To find the underlying dimensions of remotely sensed data, a commonly used technique is that of principal components analysis (PCA). As described by Richards (1986) and characterized by Fung and LeDrew (1987), the PCA transformation involves three steps:

- derivation of the variance-covariance matrix
- computation of eigenvectors
- linear transformation of the dataset

It has long been recognized that multispectral data from remote sensing scanners of the Landsat series of satellites (as well as from other scanners with similar band selections) exhibit high interband correlations and therefore may involve a considerable degree of redundancy. Thus, the uncorrelated linearly transformed components are derived from the original dataset in a manner such that the first principal component accounts for the maximum possible proportion of the variance of that dataset. The remaining components, in a descending ordered sequence, account for the maximum proportion of the unexplained residual variance. With Landsat MSS and TM data it is not uncommon to find 80+ to 90+ percent of the explained variance in the reflectance dataset to be found in the first or first plus second principal components.

Virtually all applications of PCA to remotely sensed data have been employed to assess terrestrial (vegetation, geology, etc.) targets. An excellent review of some of the more significant uses of PCA for such purposes, as well as a discussion of alternative approaches within the technique itself, is provided by Fung and LeDrew (1987). Our paper, perhaps the first of its kind, focuses on only aquatic targets, the lakes of Northeastern Ontario. The principal dataset consisted of the mean values of the seven TM bands for each of the lakes in the study area. This entire dataset was subjected to PCA as a whole or was subdivided prior to PCA using a number of categories based on selected values of DOC. All seven eigenvalues were computed for each subset of data but only the statistics for the first five principal components are listed for the illustrations below.

The eigenstructure of the TM data based on all mean pixel values for the 633 study lakes is provided in Table 3. From that table it can be seen that the visible bands TM3 and TM2 are heavily loaded on the first component (PCA-1) and the visible (blue) band TM1 is moderately loaded on that same component. The mid-infrared bands, TM7 and TM5, are heavily loaded on PCA-2, and the near-infrared band, TM4 is loaded highly on PCA-3. These three components together account for 88.2% of the overall variance of the lakes reflectance dataset. They provide evidence of the expected contrast between the spectral responses of water bodies. This is attributed to the fact that clear water bodies, for the most part, absorb infrared radiation but are highly reflective in the visible portion of the spectrum.

The visible-then-infrared sequence of components are in direct contrast to many published works on the applications of PCA in remote sensing. As illustrated by the work of Fung and LeDrew (1987), the normal expectation would be for an infrared-then-visible series of components because such work has focused on terrestrial targets. There the great variety of vegetation types results in a PCA-1 that discriminates between the high infrared reflecting targets of forest and crop land to the low infrared reflecting surfaces of plowed fields, cutovers, or lands in rural-to-urban conversion.

PCA-5, with the blue waveband TM1 loading heavily on it and contributing less than four percent to the explained variance, seems somewhat paradoxical. We have argued, as indeed many have argued, that the addition of TM1 to the Thematic Mapper is one of the strengths of that scanner for water quality analyses compared to the band selections available for the Landsat MSS or the SPOT HRV instruments. Why isn't TM1 a major contributor to PCA-1? It would appear that it acts as a controlling variable, in the sense of a partial correlation, when all water bodies are being examined. Later in this paper it will be seen that TM1 plays a much more significant role when contrasting water bodies of differing DOC.

Of great interest is the contribution of the thermal band (TM6) to the fourth most important principal component (PCA-4). While only five percent of the total variance is accounted for in this component, it does support earlier observations (Pitblado 1987a and 1987b) that clear lakes (i.e. highly reflective in the visible) tend to be cooler. It is a great pity that the TM6 spatial resolution is so coarse

(120-m). In many instances in Northeastern Ontario our study lakes are so small that the TM6 responses are too often coming from lake-lake shore mixels. It is to be hoped that future satellite scanners will provide superior thermal sensing capabilities that will enable us to more easily detect the extremely important differences of within- and between-lake temperature differences.

Table 3. Eigenstructure of all water bodies in the study area.

	COMPONENTS				
Band	PCA-1	PCA-2	PCA-3	PCA-4	PCA-5
TM3	**.88832**	.27079	.21358	.15969	.10698
TM2	**.86757**	.20129	.15921	.07654	.31566
TM7	.29288	**.85231**	.26619	.22282	.19464
TM5	.25087	**.80219**	.37254	.23630	.19157
TM4	.23081	.35513	**.89154**	.10662	.11988
TM6	-.12468	-.21443	-.09472	**-.95981**	-.09072
TM1	**.53873**	.35938	.18583	.17121	**.71878**
eigenvalues	4.51325	.94987	.71251	.37515	.25174
% variance	64.5	13.6	10.2	5.4	3.6
cum. % variance	64.5	78.0	88.2	93.6	97.2

In order to display the contrasting PCA results the lakes were subdivided into three subsets based on arbitrarily selected categories of DOC. The first subset, of 66 lakes, consisted of lakes with a DOC concentration of 2.0 mg/L or less. The actual range of DOC for this group of lakes was from 0.1 to 2.0 with a mean of 1.04 mg/L and a 0.66 standard deviation.

The second subset had a mean of 3.06 mg/L and a standard deviation of 0.52 mg/L. This group contained 88 lakes with DOC ranging from 2.1 to 4.0 mg/L. The final set of 45 lakes ranged in DOC concentration from 4.1 to 6.5 mg/L. Here, with slightly greater within-group variation as expressed by a standard deviation of 0.71, the average concentration was 5.13 mg/L. Each of these subsets of lakes was subjected to PCA using the same routine as was employed for the entire dataset. The numerical results of those analyses are tabulated in Tables 4 to 6.

One would anticipate that a group of lakes with little or no ($<=$ 2.0 mg/L) dissolved organic carbon would be highly reflective, especially in the visible bands of the Thematic Mapper. This is in fact the case as illustrated in the summary Table 7, noting that the highest spectral response occurs in TM1. The eigenstructure of this group (Table 4) of lakes reflects that fact with TM1 explaining little of the variance of that dataset. There the visible bands TM1 (blue) and TM2 (green) load most heavily only on the third and fourth components, respectively. In Northeastern Ontario, to explain the variance in these highly reflective lakes one must look to subtle differences in the near- and mid-infrared bands, particularly. As

Table 4. Eigenstructure of lakes in the study area with DOC less than or equal to 2.0 mg/L.

Band	COMPONENTS PCA-1	PCA-2	PCA-3	PCA-4	PCA-5
TM4	**.81683**	.29187	.29963	.34576	.09168
TM3	**.77896**	.24608	.27155	.35891	.29471
TM5	**.77052**	.39471	.28362	.28125	.23271
TM7	**.58217**	.39484	.32558	.27416	.56904
TM6	-.29191	**-.89631**	-.25985	-.15238	-.14342
TM1	.32203	.32169	**.82727**	.28793	.15914
TM2	.48070	.19454	.34352	**.76570**	.16276
eigenvalues	5.67212	.53282	.37403	.18416	.14453
% variance	81.0	7.6	5.3	2.6	2.1
cum. % variance	81.0	88.6	94.0	96.6	98.7

Table 5. Eigenstructure of lakes in the study area with DOC greater than 2.0 but less than or equal to 4.0 mg/L.

Band	COMPONENTS PCA-1	PCA-2	PCA-3	PCA-4	PCA-5
TM2	**.77907**	.30075	.30989	.34338	.23997
TM1	**.68201**	.30923	.38387	.30405	.35425
TM6	-.25767	**-.87815**	-.26850	-.21168	-.19001
TM7	.35572	.34922	**.77449**	.25665	.27232
TM5	.39518	.42178	**.50248**	.37895	.28766
TM4	.47185	.31550	.30813	**.70608**	.26117
TM3	.44330	.32464	.40256	.30480	**.65211**
eigenvalues	5.90919	.41308	.24794	.16718	.12757
% variance	84.4	5.9	3.5	2.4	1.8
cum. % variance	84.4	90.3	93.9	96.2	98.1

Table 6. Eigenstructure of lakes in the study area with DOC greater than 4.0 but less than or equal to 6.5 mg ..

Band	COMPONENTS PCA-1	PCA-2	PCA-3	PCA-4	PCA-5
TM1	.85530	.20133	.31260	.20102	.25944
TM3	.80799	.23296	.31487	.26436	.25207
TM2	.79851	.14144	.42693	.29174	.22051
TM6	-.16677	-.95785	-.11389	-.14902	-.13929
TM4	.45945	.15411	.82685	.20768	.19484
TM5	.47206	.34172	.31388	.68498	.30377
TM7	.51248	.31080	.28998	.31057	.67806
eigenvalues	5.44773	.78674	.28567	.23464	.14303
% variance	77.8	11.2	4.1	3.4	2.0
cum. % variance	77.8	89.1	93.1	96.5	98.5

Table 7. Mean values (haze-corrected digital numbers) for each of the seven TM bands for each of the three DOC groups.

DOC GROUPS	Thematic Mapper Bands TM1	TM2	TM3	TM4	TM5	TM6	TM7
1	11.85	5.13	4.74	9.04	6.84	121.58	2.98
2	7.70	4.35	4.68	8.20	6.82	121.81	2.95
3	6.66	3.82	4.45	7.50	6.17	121.84	2.92

Table 8. Classification results using discriminant analysis. This analysis employed the three DOC-defined groups and the seven TM bands. Included in this table are the predicted group memberships of the 434 lakes which where not included in the predefined groups.

DOC Group	No. of CASES	Predicted Group Membership 1	2	3
1 (Low DOC)	66	52 78.8%	13 19.7%	1 1.5%
2	88	6 6.8%	47 53.4%	35 39.8%
3 (High DOC)	45	2 4.4%	8 17.8%	35 77.8%
UNGROUPED	434	31 7.1%	143 32.9%	260 59.9%

much as 81% of the total variance is explained by those TM bands that centre around the near-infrared (TM4 and TM5), including the visible band TM3 (red) which tends to overlap with the near-infrared. A minor contribution to this component is provided by the mid-infrared band (TM7). This latter band is really of little significance as suggested by the mean values of TM7 in the three DOC groups of lakes (Table 7) which differ only by a maximum of .06 haze-corrected digital numbers.

Slightly more than seven percent of the variance within low DOC lakes is explained by the PCA-2 where the thermal band (TM6) is loaded heavily. In fact this band shows up on the second component of all three lake groups defined by the DOC categories, explaining from 5.9% (Table 5) to 11.2% (Table 6) of the total variance within the respective lake groupings. Frankly, this is somewhat surprising. While we argue the value of this thermal band in looking at water bodies in Northeastern Ontario, it is clear from Table 7 that the digital numbers of TM6 vary little from one group of lakes to another. This spectral response and its apparent ability to discriminate between various lake types will require further investigation.

Turning to the other extreme of the DOC groups with measured concentrations greater than 4.0 but less than 6.5 mg/L, we see that the eigenstructure (Table 6) is the reverse of that for the low DOC group. There the visible bands (TM1, TM2, TM3) are loaded highly on PCA-1 and it is the near- to mid-infrared bands (TM4, TM5, TM7) that occupy positions of heavy loadings on the third and subsequent components. And again the thermal band is the most significant variable to load on PCA-2.

We attribute this reversal of the loadings to the fact that these are the most coloured of the lakes in the entire dataset. They would be expected to reflect more in the infrared in comparison to the visible bands. As suggested by the figures provided in Table 8, these comparisons must be made mostly in relative and not absolute terms. Thus, in the examination of the eigenstructure (Table 7) of the high DOC lakes we see that if these lakes are to be differentiated within that group one would have to examine the visible bands which explain 77.8% of the variance.

Our middle group, with DOC between 2.0 and 4.0 mg/L, is most similar to the higher DOC lakes. Again (Table 5), the sequence of components and the respective loadings of the TM bands are: PCA-1, visible bands; PCA-2, thermal band; and the infrared bands loaded on the third and subsequent components. The visible band that occurs very close to the infrared, TM3, plays an insignificant role.

We have conducted an experiment to see whether classification using discriminant analysis (SPSS Inc., 1988) would maintain the structure of these DOC groups. Summary results are provided in Table 8. That table basically preserves the interpretations suggested for the eigenstructures of the three DOC lake groupings. Close to eighty percent of the lakes in the first and third DOC groups are correctly classified, but the lakes of the second group are spread primarily between the middle (second group) and the high (third group) DOC lakes. On the canonical discriminant functions that were generated for this analysis: TM1 and TM6 were the only bands with significant correlations on the first function, accounting for 96.1% of the variance (P < 0.001). All of the other TM bands were correlated, if at all, with the second discriminant function.

Given the fact that our DOC group limits were arbitrarily defined and that we have not employed any numerical transformations of the TM data as is so often the case for water quality analyses (Scarpace et al., 1979; Verdin, 1985; Lathrop, Jr. and Lillesand, 1986), we are not displeased with these preliminary results. Indeed, it is interesting to note that of the 434 lakes identified in Table 8 as Ungrouped, only two have DOC measurements that we can employ for comparison purposes. These two lakes have DOC concentrations of 9.7 and 10.5 mg/L, respectively. The discriminant classification showed that their membership of highest probability would be group 3. Exactly as would be expected! For comparison purposes with Table 7, the band means for these two lakes are (from TM1 to TM7, respectively): 4.62, 2.10, 3.25, 5.90, 4.48, 122.83, and 2.01.

This summary, interim paper has described some of the basic, underlying structure of the Landsat-5 Thematic Mapper spectral responses of lakes in Northeastern Ontario. The description and discussion has been provided for the entire, current dataset as well as subsets based on DOC lake groupings. Future work will be undertaken for subsets of the sampled lakes where the groupings are based on other optically significant water quality parameters and predictions made for those parameters for all lakes in the study area that can be discriminated ("located") by the TM-5 sensor.

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B18

Relationship of Mercury Levels in Sportfish with Lake Sediment and Water Quality Variables.

Christopher Wren, B.A.R. Environmental, Nicholas Beaver Park,
R.R. #3, Guelph, Ontario, N1H 6H9 (519) 824-5000

SUMMARY

Tissue mercury levels in smallmouth bass and walleye were weakly correlated with background sediment mercury levels. Mercury levels in lake trout were not correlated with background sediment mercury concentrations.

Results suggest that geological mercury levels do not account for differences in fish mercury levels observed between lakes. Mercury concentrations in standard size smallmouth bass (31 cm) and walleye (41 cm) were negatively correlated with water quality variables reflecting water hardness and acidity. Therefore, fish of these species in low pH lakes tend to have elevated mercury levels relative to circumneutral lakes. Mercury concentrations in standard size (44 cm) lake trout were positively correlated with dissolved organic carbon and lake area.

There was a very high correlation between standardized mercury concentrations in smallmouth bass and walleye, and between smallmouth bass and lake trout. The development of interspecies correlations could provide a useful management tool.

The results of this study support the premise that a number of lake physical, chemical and biological variables simultaneously influence mercury accumulation and availability within lakes.

OBJECTIVES

The primary objective of this study was to examine the relationship between mercury levels in sportfish and natural sediment mercury content of lakes.

1. INTRODUCTION

It is well established that mercury levels in fish even from waters remote from direct pollution vary significantly between lakes, but the overall factors determining spatial variability remain obsure. A number of studies have reported that fish mercury levels are elevated in low pH lakes, suggesting that lake acidification is a factor affecting mercury uptake in fish (Brouzes et al 1977: Suns et al 1987: Wren and MacCrimmon 1983: Bjorklund et al 1984). A variety of other biotic and abiotic variables are also known to influence mercury uptake in fish (eg. Richman et al 1988; Verta 1984; 1985).

Mercury occurs naturally in combination with sulphide (HgS) known as cinnibar (Boyle 1974). The mercury content of ores generally increases with increasing zinc content, indicating that most mercury is present as a constituent of sphalerite (ZnS), and is much less abundant in copper concentrates (Maclatchy and Jonasson 1974).

Bedrock geology and soil type are known to have a profound influence on the chemical composition of many plant species, which in turn can directly affect the levels of certain elements (e.g. Se, Cu, Mo) in terrestrial animals. However, the relationship between bedrock geology and mercury burdens in fish has not been seriously investigated.

Some preliminary comparisons of fish mercury levels with sediment mercury levels have failed to establish a simple relationship between these two variables (e.g. McFarlane and Franzin 1980). However, many of those studies were based on a relatively small number of lakes. Bjorklund et al. (1984) reported a relationship between fish mercury burdens and surface sediment levels in Sweden. Hakonson (1980) developed a simple model to predict the mercury content of pike in Sweden based on surface sediment mercury content, water pH and a lake productivity index.

Detailed lake sediment geochemical data, including mercury, are available for areas of Ontario through the Geological Survey of Canada. These data were used to investigate the potential role of natural geological mercury levels in affecting mercury levels in fish. Shilts (1982) states that metal enrichment (in biota or sediments) in certain areas cannot be casually attributed to man's activities without knowledge of bedrock and sediment geochemistry.

3.0 METHODS AND MATERIALS

3.1 Data Collection

3.1.1 Lake Sediment Geochemistry

Lake sediment geochemical data were obtained from the GSC (Geological Survey of Canada), Energy Mines and Resources in Ottawa.

The GSC data were collected as part of reconnaissance surveys designed to gather a single index sample from a large number of lakes over a wide area. Both sediment and water samples are collected from each lake visited. Lake sediment samples were collected from a helicopter during the open water period. Samples were collected from the deepest portion of the lake. The theory is that a midlake sample is the most representative of a watershed erosional product.

3.1.2 Provincial Water Quality and Fish Mercury Data

The Ontario Ministry of the Environment and the Ontario Ministry of Natural Resources have jointly developed databases incorporating mercury levels at a standard fish length, lake physical characteristics and water quality data for a number of lakes in Ontario. Databases were available for smallmouth bass, lake trout, and walleye. Mercury levels at standard length were available for the following number of lakes: Smallmouth bass, 91; Lake trout, 91; Walleye , 255.

All mercury concentrations in fish tissue were analyzed by the Ontario Ministry of the Environment (OME 1981).

Individual lakes were chosen on the basis that at least 10 fish were sampled for mercury from that lake, and that there was a statistically significant correlation ($p < 0.05$) between log mercury and total fish length (McMurtry 1986). To compare mercury levels between lakes without a length bias, the average mercury concentration was predicted for a hypothetical fish of a standard length. The following standard lengths were utilized for the three species: Smallmouth bass, 31 cm; Lake trout, 44 cm; and Walleye, 41 cm.

Water quality data from the Provincial Acid Sensitivity database were incorporated with the fish mercury data set. Sediment geochemical data were merged with the water chemistry and fish mercury data for corresponding lakes.

3.2 Data Analysis

Summary statistics, Pearson correlation coefficients, residuals and stepwise multiple regression analysis were conducted using SPSS/PC+.

The $\log_{10}$ of standardized fish mercury was used as the independent variable for all regression analysis. Some water quality variables were transformed for regression analysis as described by McMurtry (1986). Sediment mercury concentrations were normal so the data were not transformed. Subsets of lake variables were entered as independent variables in stepwise regression analysis. Entering subsets of independent variables reduced two fundamental problems in multiple regression analysis: a) multicolinearity, and b) missing values.

4. RESULTS

4.1 Smallmouth bass

The mean standarized fish mercury concentration for a 31 cm bass was 402 ng/g (range 132-943 ng/g). The mean pH of these lakes was 6.97 (range 5.6-8.2). The mean alkalinity was 33.9 mg/L. The mean sediment mercury concentration was 99 ng/g (range 5-180 ng/g).

The standardized log fish mercury concentration was significantly correlated with a number of sediment and water quality variables. Fish mercury was positively correlated with sediment mercury concentrations ($r = 0.31$, Figure 1), and negatively correlated with lake pH ($r = -0.52$; Figure 2).

The mean mercury concentration in smallmouth bass in lakes with pH $\geq$ 6.5 was 353 ng/g ($n = 49$), compared with 545 ng/g in lakes with pH $<$ 6.5 ($n = 17$).

4.2 Lake Trout

The mean standardized mercury concentration in lake trout was 303 ng/g ($n = 41$ lakes, range = 70 - 1033 ng/g). The mean sediment mercury concentration in lake trout lakes was 138 ng/g.

The mean pH of the lake trout lakes was 6.4. The average pH of lake trout lakes was substantially lower than the average pH of either smallmouth bass or walleye lakes.

Fish mercury concentration was not significantly correlated ($p > 0.05$) with lake pH ($r = 0.24$) or sediment mercury concentration ($r = -0.10$). The relationship between mercury concentration in lake trout and sediment mercury concentration is illustrated in Figure 3.

4.3 Walleye

The mean standardized mercury concentration in walleye was 517 ng/g (n= 44 lakes, range = 128-2216). The mean sediment mercury concentration was 88 ng/g (range 10-188).

The average pH of the walleye lakes was 7.30. The average pH was similar to smallmouth bass lakes but the range was not as great. For example, only 2 of the 44 walleye lakes had pH < 6.5.

Stepwise multiple linear regression analysis consistently chose conductivity as the single best predictor of log fish mercury concentration.

4.4 Interspecies Correlations

There was a good correlation of standard mercury concentrations between species among overlapping lakes. Figures 4 illustrates the relationships between smallmouth bass and walleye mercury concentrations.

5.0 DISCUSSION

The results suggest that background sediment mercury levels were significantly correlated with mercury levels in smallmouth bass and walleye. However, the background sediment mercury levels did not explain the differences observed in fish mercury levels between lakes.

The average background sediment mercury levels in this study agree very closely with other studies that suggest the background mercury concentration in lake sediments is approximately 100 ng/g (Forstner and Whittman 1981; Cahill and Shimp 1984; Bjorklund et al 1984; Evans 1986).

The results of this study and those of Johnson (1987) and Bjorklund (1984) suggest that surface sediment mercury concentrations may be better than background sediment levels as an indicator of mercury availability, and fish mercury levels within a lake. However, since other factors such as water quality have an obvious influence on fish mercury levels, it is becoming apparent that fish mercury levels cannot be accurately predicted by a single environmental variable.

Mercury levels in smallmouth bass and walleye were highly correlated with variables reflecting water hardness and acidity. Calcium and conductivity were generally better single predictors of fish mercury than lake pH. This may be partially attributed to the quality and variability of lake pH data, and also to greater direct influence of calcium than pH on mercury uptake.

Water hardness and lake pH likely influence mercury uptake and availability simultaneously within a lake. Rodgers and Beamish (1983) reported that the efficiency of methyl mercury uptake by rainbow trout was much greater in softwater (30 mg/l as CaCO3) compared with hardwater (385 mg/l as CaCO3). The mechanisms to account for this effect may be increased gill permeability at low Ca levels (Spry et al 1981) or competition between metals and Ca for cellular binding sites (Zitko and Carson 1976).

Reduced lake pH may increase the bioavailability of mercury by stimulating bacterial methylation from the sediments (Xun et al 1987). Thus, a combination of increased production and uptake efficiency could explain elevated mercury levels in fish from low pH lakes.

Negative correlations between lake pH and/or alkalinity and mercury content of fish in Ontario have now been demonstrated for yearling yellow perch (Suns et al 1980), pumpkinseed sunfish (Wren and MacCrimmon 1983), smallmouth bass (McMurtry 1987; Suns et al 1987) and now walleye (this study). Of these, the walleye lakes had the greatest geographical distribution in the province.

Mercury concentrations in standard length lake trout were highly correlated to DOC and lake area, but not water hardness or acidity variables. Dissolved organic carbon was also selected in conjunction with calcium as predictors of mercury levels in smallmouth bass. Studies in Sweden and Finland have also noted a positive correlation between fish mercury levels and the humic content of water (Verta 1984; 1985; Lindqvist et al 1986). It is suggested that the humic substances act as both an energy source and source of mercury for the methylating bacteria (Verta 1985). Bodaly and Hecky (1984) suggest that increased availability of organic material accounts for elevated fish mercury levels in new hydroelectric reservoirs.

It is notable that the average mercury concentration in a 41 cm walleye (517 ng/g) is above the recommended safe level of mercury in fish for unlimited consumption. A length of 41 cm corresponds to the average length of walleye sampled in the sportfish contaminant monitoring program (Scheider pers commun).
The demonstrated relationship of mercury concentrations between species is significant. This is the first study to document a relationship between mercury levels in sportfish from such a large number of lakes. A relationship between mercury levels in

different fish species has great potential as a management tool both for predicting mercury levels in other species, and for designing fish contaminant monitoring programs.

The results indicate that background sediment mercury levels do not account for the observed differences in mercury levels in fish between lakes. The differences may often be explained by water quality, expecially DOC, pH and alkalinity. Therefore, the factors and conditions affecting these variables in Ontaro lakes will also influence mercury availability and uptake in fish. These variables will act simultaneously on mercury uptake and availability, and the net effect from individual variables will differ between species and lakes.

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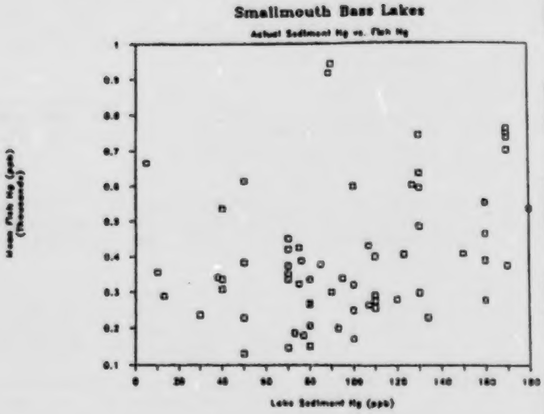

Figure 1. The relationship between standardized mercury levels in smallmouth bass and lake sediment mercury level.

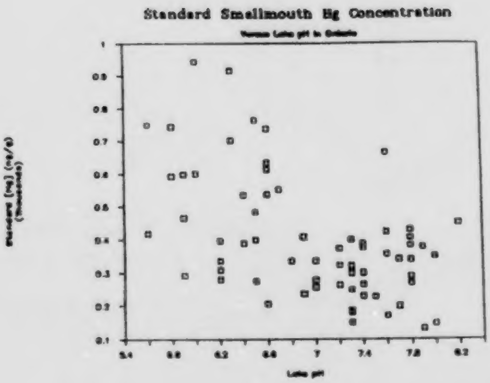

Figure 2. The relationship between standardized mercury levels in smallmouth bass and lake pH in Ontario.

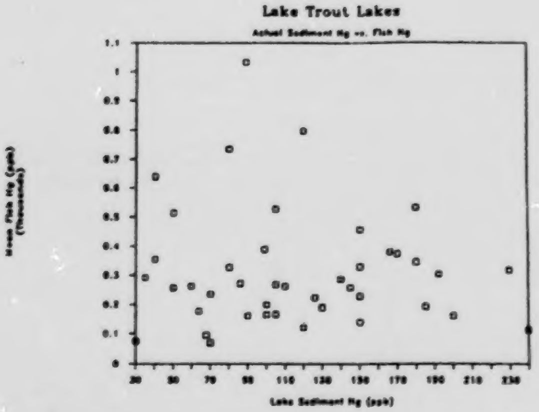

Figure 3. The relationship between standardized mercury concentration in Lake trout and lake sediment mercury levels.

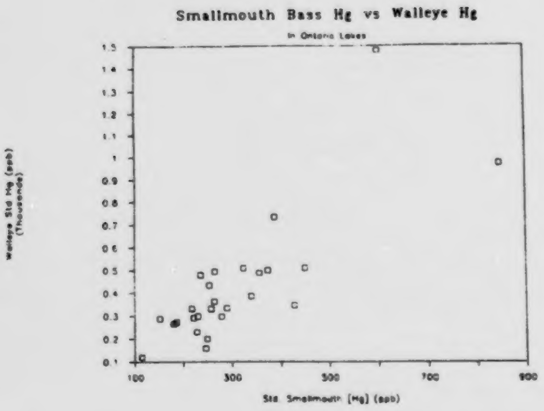

Figure 6. Standardized mercury concentrations in walleye in relation to standardized mercury concentrations in sma-lmouth bass in Ontario lakes.

B19

TREND ANALYSIS PROCEDURES
FOR WATER QUALITY TIME SERIES

by

A. Ian McLeod
Department of Statistical and Actuarial Science
The University of Western Ontario, London, Ontario

Keith W. Hipel
Departments of Systems Design Engineering
and Statistics and Actuarial Science
University of Waterloo, Waterloo, Ontario

Byron Bodo
Water Resources Branch, Ontario Ministry of the Environment
and Adjunct Professor in the Department of
Statistical and Actuarial Sciences
The University of Western Ontario

ABSTRACT

The overall objective of the study is to develop graphical and statistical trend analysis procedures for use with the water quality time series obtained from Ontario's Provincial Water Quality Monitoring Network (PWQMN). Trend analysis is required for alerting authorities about water quality degradation so that appropriate corrective action can be taken and evaluating the performance of pollution abatement schemes. In order to detect visually increasing or decreasing trends, a range of exploratory data analysis techniques are being applied to PWQMN data sets. The graphical methods include time series plots, box and whisker graphs and a smoothing technique called robust locally weighted regression. Special types of nonparametric tests are being refined and developed for rigorously testing for the presence of trends. These tests will also be applied to PWQMN water quality time series.

INTRODUCTION

Water quality and other kinds of environmental time series often possess characteristics which do not allow the series to be easily analyzed using statistical techniques [3 to 7, 9, 11, 13]. One of the major problems with these time series is that there are often many missing data points among which there may be long periods of time for which no observations were taken. Water quality data may be non-normally distributed and follow a distribution which is usually positively skewed. In addition, the data are often censored by only listing measurements below a certain level as being "less than" or measurements above a specified level as being "greater than". For instance, concentration values for toxic compounds, metals or organic compounds which fall below the limits of detection for certain chemical tests are reported simply as less than the limits of detection. Seasonality effects contained in a sequence of measurements over the years can cause cyclic patterns to appear in a graph of a given water quality variable. When there are many different water quality variables interacting with one another in the presence of varying flow rates, the multivariate effects of the interactions can be quite complex. As a further major complication, one or more external interventions may significantly affect the stochastic manner in which a series behaves and thereby create a variety of trends. Because of the foregoing and other reasons, environmental data are often quite "messy".

In order to extract an optimal amount of information from messy environmental data, a systems design approach to data analysis can be followed. As proposed by Tukey [14] and demonstrated by authors such as McLeod et al. [11] and Hipel et al. [5] using water quality data, the two major steps in a statistical study consist of exploratory and confirmatory data analysis. The objective of the exploratory data analysis stage is to employ simple graphical and numerical techniques to discover important patterns and statistical characteristics such as the presence of trends. The purposes of the confirmatory data analysis stage are to confirm statistically in a rigorous fashion the presence or absence of certain properties in the data. Depending upon the quantity and quality of the data being analyzed, appropriate graphical, parametric and nonparametric techniques can be employed as exploratory and confirmatory data analysis tools.

Both exploratory and confirmatory data analysis methods are used in this study for detecting and modelling trends contained in water quality time series obtained from Ontario's Provincial Water Quality Monitoring Network (PWQMN). In the next section, representative results are presented for demonstrating how some different kinds of graphs can be used as exploratory tools for visually detecting trends and other statistical properties in water quality series. Following this, it is explained how a number of nonparametric tests are being developed and employed for finding trends in messy water quality time series.

GRAPHS

Different types of graphs are available for use as exploratory data analysis tools for detecting trends in a data set. Table 1 lists some of these graphs along with brief descriptions of their purposes and references containing detailed explanations about the techniques. Applications of some of these graphical methods to water quality time series are presented in publications by authors such as El-Shaarawi and Kwiatowski [3], McLeod et al. [11], Hipel et al. [5] and Hipel [4]. Below, representative results are given for the cases when two types of the graphs in Table 1 are used with a PWQMN data set.

Table 1.
Graphs for use in Trend Analysis at the Exploratory Data Analysis Stage

Types of Graphs	Purposes	References for Descriptions
Time Series Plot	Detect trends and other statistical characteristics.	Tukey [14]. Most statistical textbooks.
Box and Whisker Plots	Graphically summarize important statistics of data for each season of the year. Compare plots before and after the intervention.	Tukey [14, Ch. 2] McGill et al. [10]
Star Symbol Plot	Display how medians and other statistics change across seasons and years.	Chambers et al. [1]
Tukey Blurred Smooth	Trace trends	Tukey [14, Ch. 7] McNeil [12]
Robust Locally Weighted Regression Smooth (RLWRS)	Trace the general shape of trends in a time series plot	Cleveland [2]

Removal of the Effects of Flow

The statistical properties of a given water quality variable are often dependent upon riverflows, as well as other physical factors. After removing the statistical effects of flows contained in the water quality time series, one can check for the presence of trends in the residual or filtered water quality time series. One approach for removing flow levels, represented by x_i, from the water quality variable, y_i, is to employ the cubic regression analysis equation given by

$$y_i = \alpha + B_1 x_i + B_2 x_i^2 + B_3 x_i^3 + e_i \qquad (1)$$

where α is the level parameter; B_j, $j = 1,2,3$, are the regression parameters; and e_i represents the residual series. Subsequently, exploratory and confirmatory data analysis tools can be used for detecting and modelling trends in the residual series in (1). Furthermore, when deemed necessary, one may wish to take natural logarithms or some other kind of data transformation of the y_i and/or x_i series before performing the regression analysis.

On the Grand River at Dunnville, Ontario, there are 1700 average daily values of total phosphorous (mg/l) available from 1972 to 1985, as well as average daily riverflows (m^3/s). When equation (1) is used to regress the logarithmic total phosphorous observations upon the logarithmic flows, all of the regression parameters are significantly different from zero. Consequently, in the applications the residuals of the regression analysis are employed.

Box and Whisker Graphs

A box and whisker graph is based upon what is called a 5-number summary [14, Ch. 2]. For a given data set, the 5-number summary consists of the smallest and largest values, the median, and the 0.25 and 0.75 quantiles, which are called hinges. When the data are ranked from largest to smallest, the first data point is the smallest value while the last entry is the largest. When examining a seasonal time series, such as monthly or quarterly data, it is instructive to calculate a 5-number summary plus certain types of extreme values for each season. A convenient manner in which to display this information is to plot a "box and whisker" diagram for each season or month.

Figure 1 displays a notched box and whisker plot for each month of the residuals for the total phosphorous series at Dunville, Ontario. For a given month, the upper and lower ends of a notched rectangle represent the two hinges and the thick line drawn horizontally in the rectangle is the value of the median. The width of each box is proportional to the number of data points in the corresponding season. Excluding extreme values, the minimum and maximum values for a particular month are the end points of the lines or "whiskers" attached to the rectangle or box. A special type of extreme value called a far-out value [14, Ch. 2] is indicated by the points plotted above and below the whiskers in a given season. The notch on both sides of the median allows one to compare medians across months or seasons. If, when comparing two months, the notches overlap, one can argue that the medians for those months are not significantly different from one another at the 95% confidence level. In subsequent analyses, one may wish to join similar months together as a single season in order to provide results which are simple and easier to understand.

Figure 2 shows a graph of the average residual series for total phosphorous when the 12 monthly series are combined as five seasonal residual series. The legend explains how consecutive months are joined together. For example, NDJ means the residuals for November, December and January are considered as a single season across all the years. The five time series plots in Figure 2 for the total phosphorous residuals, clearly portray a downward trend in the earlier 1970's which then levels off from the mid-1970's onwards.

Nonparametric Tests

Various kinds of graphs are used as exploratory data analysis tools for visually finding trends in a time series. At the confirmatory data analysis stage, statistical tests can be employed for detecting trends and perhaps also estimating their magnitudes. In order to lessen the number of underlying assumptions required for testing a hypothesis such as the presence of a specific kind of trend in a data set, researchers developed nonparametric tests [8]. Nonparametric tests were developed for use in environmental impact assessment because scientists were concerned that the statistical characteristics of messy environmental data would make it difficult to use the parametric procedures [3 to 7, 9, 13].

In this study, a number of nonparametric tests are being refined for application to PWQMN water quality time series. One parametric trend test that is currently being applied to the data is a version of the seasonal Mann-

Kendall test [6,7,13]. Kendall's partial rank correlation test [8] is also being further developed so it can be applied to water quality time series. Instead of using a parametric procedure such as the regression analysis method in (1) to remove flow effects in a water quality series, one can employ the partial rank correlation approach to both partial out the flows and test for the presence of a monotonic trend over time in the water quality series being considered.

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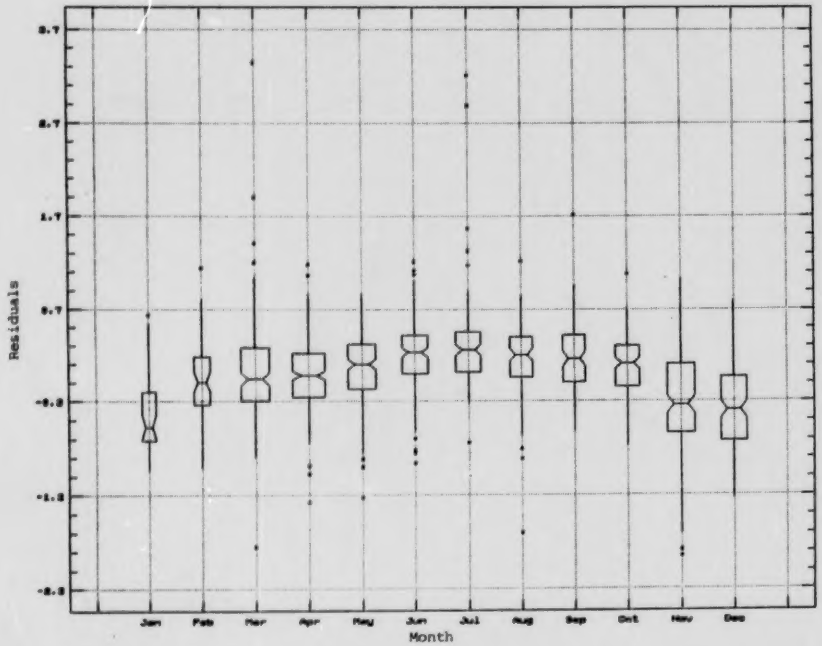

Figure 1. Box and Whisker Plots for the Residuals of the Total Phosphorous Measurements on the Grand River at Dunnville, Ontario.

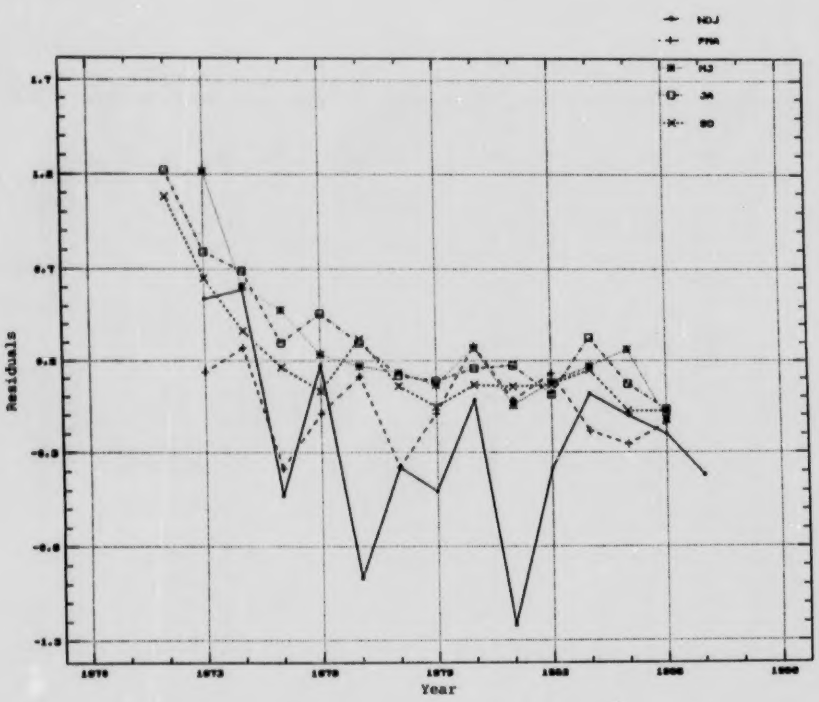

Figure 2. Seasonal Residual Series of the Total Phosphorous Observations on the Grand River at Dunnville, Ontario

B20

Use of a Bromobenzoate for Cross-adaptation of Anaerobic Bacteria In Lake Ontario Sediments for Degradation of Chlorinated Aromatics

Martina Urbanek[1], Tatiana Strycek[1], Campbell Wyndham[2] and
Morris Goldner[1]

[1]Department of Microbiology, University of Toronto, Toronto,
Ontario, Canada M5S 1A8
[2]Department of Biology and Environmental Laboratories, Carleton
University, Ottawa, Ontario K1S 5B6

INTRODUCTION

In surveys of Toronto waterfront sediments, Persaud et al. (1985) disclosed that muds in boat slips of the Inner Harbour and in Toronto Island waterways had elevated levels of total volatile residues, solvent extractables, oil and grease, and PCB's. Many such compounds were originally believed to be recalcitrant. Within the past few years several research groups have found that chlorinated aromatic compounds can be decomposed in the laboratory by stringently anaerobic bacterial consortia present in lake sediments (Horowitz et al. 1982, 1983; Suflita et al. 1982, 1983; Shelton & Tiedje 1984a, 1984b; Tiedje et al. 1986).

In the light of these findings, studies were undertaken in order to investigate the potential of sediment microorganisms for anaerobic degradation of halogenated pollutants. Sediments were collected from sites along the Toronto waterfront in Lake Ontario (Toronto, Ontario, Canada). These were anaerobically incubated with monohalogenated benzoates. Following adaptation, as measured by the rate of substrate disappearance, the sediments were subsequently incubated with polysubstituted benzoates. Cross-adaptation to these complex aromatics was assessed by high pressure liquid chromatography (HPLC). The findings demonstrate a potential for breakdown of halogenated contaminants by anaerobic microorganisms found in Lake Ontario sediment. This points to the feasibility of applications of preadapted microbial consortia for anaerobic degradation of xenobiotics under controlled conditions.

MATERIALS AND METHODS

Sediment collection

Sediment samples were collected in Lake Ontario from sites located along the Toronto waterfront by Ponar grab sampler, and kept in glass jars (0.5 l capacity) at 4°C in the dark under anaerobic conditions until further use. The sampling sites were characterized by organic carbon-rich sediments which were anaerobic at all depths.

Preparation of test medium

Revised Anaerobic Mineral Medium (RAMM) was used as suspending medium (Shelton & Tiedje, 1984a). The medium contained phosphate buffer (pH 7.0), mineral salts, trace metals, vitamins, reducing agent (0.125% L-cysteine.HCl-$Na_2S.9H_2O$, pH 10), and a redox indicator (0.1% resazurin). Preparation and transfer of media and inocula were carried out in an anaerobic hood with protective eyewear and gloves. Hungate technique and apparatus (Hungate, 1968), were used for anaerobic gassing of flasks and for preparation of oxygen-free gases and media.

Chemicals

3-bromobenzoate (3-BrBZ) and 3-chlorobenzoate (3-ClBZ) were obtained from Sigma Chemical Co., St. Louis, MO.; 3,5-dichlorobenzoate (3,5-diClBZ) was supplied by Fluka Chemical Corp., Ronkonkoma, NY.; and, 4-amino-3,5-dichlorobenzoate (4-amino-3,5-diClBZ) was obtained from Pfaltz and Bauer Research Chemical Division, Waterbury, CT.

Water insoluble test compounds were first dissolved in 1M NaOH. Appropriate amounts were dispensed through a disposable membrane filter (0.45 um, Millipore Ltd., Montreal, Que.).

Cross-adaptation experiment

Collected sediment samples in volumes of 500 mL were anaerobically transferred to 2-l Erlenmeyer flasks and then suspended in RAMM in a 1:2 ratio. The flasks were sealed with thick butyl rubber stoppers. The stoppers were secured in place with tape and equipped with a needle attached to Nalgene (Nalge Co., Division of Sybron Corp., Rochester, NY.) tubing trapped in a test tube filled with water to maintain atmospheric pressure in the head space of the flasks. The substrates, 3-ClBZ and 3-BrBZ, were added to the sediment slurry in the flasks at concentrations in the range of 0.6 - 1.2 mN. Incubation was carried out in the dark at room temperature. Samples of sediment pore water were taken from flasks at regular time intervals, passed through a 0.45um Millipore disposable membrane filter, and stored at -10^{o} C for HPLC analysis. Substrate depletion was monitored in a Waters Millipore system (Waters Division of Millipore (Canada) Ltd., Mississauga, Ont., Waters 501 HPLC pump, Novapak C_{18} column, 50% methanol/1% acetic acid as solvents , UV absorbance detection at 254 nm, 10 ul sample loop). Once total substrate depletion occurred, the same substrate (0.6 - 1.2 mM) was added again. The sediment consortium was considered to be adapted after a repeated addition showed the substrate to be degraded without a lag period or with a much shortened lag period and with a noticeably diminished time for its complete depletion.

For cross-adaptation experiments, the sediments previously adapted to a monohalogenated substrate were incubated with a polyhalogenated substrate (0.6 - 1.2 mM), 3,5-diClBZ or 4-amino-3,5-diClBZ. Autoclaved (30 min, 10 lbs/sq. in.) sediments served as controls and were incubated under the same conditions as test flasks, with substrate concentrations monitored by HPLC.

RESULTS AND DISCUSSION

The ability of sediment microorganisms to adapt and cross-adapt to halogenated compounds is naturally of great interest, considering the problem that these types of chemicals pose to our freshwater ecosystems. The cross-adaptation experiments provided some insight in this direction.

HPLC analysis revealed that lag periods in non-adapted sediments were much shorter for 3-BrBZ than for 3-ClBZ; but, once degradation commenced, it appeared to be quite rapid for both substrates (Fig. 1). Subsequent additions of these substrates to the same sediment resulted in a much shortened lag period (Fig. 1) while substrates were depleted in a similar pattern, both initially and following adaptation (Table 1).

Previous experiments (Strycek et al., 1987) involving the monitoring of gas production had revealed that both 3,5-diClBZ and 4-amino-3,5-diClBZ were degraded very slowly, even after 12 weeks of incubation, with a minimal gas production, as compared to simpler aromatic compounds (e.g. 3-BrBz or 3-iodobenzoate). However, organisms adapted to 3-ClBZ required four to five weeks for 3,5-diClBZ depletion. Organisms adapted to 3-BrBZ were able to completely deplete the 3,5-diClBZ in 3 weeks. The amino derivative, 4-amino-3,5-diClBZ was only partially degraded by organisms adapted to 3-ClBZ even after 16 weeks; but, when the organisms were adapted to 3-BrBZ, they were able to completely degrade 4-amino-3,5-diClBZ in six weeks (Figure 2, Table 2).

The results from the different sediment sampling sites examined (six in total) were pooled together in order to provide representative data from which trends could be determined. The cross-adaptation showed that respectively 3,5-diClBZ and 4-amino-3,5-diClBZ were degraded more rapidly in sediments previously adapted to 3-BrBZ than in those adapted to 3-ClBZ or in non-adapted ones. Thus, pre-adaptation to 3-BrBZ may be an effective measure in inducing breakdown of the polyhalogenated derivatives.

The feasibility of inducing biologically mediated breakdown of complex halogenated aromatics suggests possible practical applications. A wider range of substrates should be tested in order to establish the generality of observations in this report. Given this further progress, adapted anaerobic sediment cultures might be usable either in in situ treatment of contaminated anaerobic sediments or in controlled wastewater treatment systems.

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Table 1. Comparison of average times for substrate depletion in adapted and non-adapted sediments

Table 2. Average comparative times for substrate depletions in cross-adapted sediments

Tables

Table 1. Comparison of average times[a] for substrate depletion in adapted and non-adapted sediments

substrate	3-BrBZ	3-ClBZ
time required for initial substrate depletion	7 weeks	15 weeks
time required for subsequent substrate depletion	4 weeks	6 weeks

[a] Data pooled for six sampling sites on the Toronto Waterfront.

Table 2. Average comparative times[a] for substrate depletions in cross-adapted sediments

adapted to:	3-BrBZ	3-ClBZ
cross-adapted to		
3,5-diClBZ	3 weeks	4.5 weeks
4-amino-3,5-diClBZ	6 weeks	16+ weeks

[a] Data pooled for two (3,5-diClBZ) and four (4-amino-3,5-diClBZ) sampling sites on the Toronto waterfront. Sediments initially adapted to 3-BrBZ and 3-ClBZ.

__Legend for Figures__

Figure 1. Substrate depletion in sediment slurries from a typical sampling site on the Toronto waterfront.
● : 3-ClBZ breakdown in non-adapted sediments
o : 3-ClBZ breakdown in adapted sediments
△ : 3-BrBZ breakdown in non-adapted sediments
▲ : 3-BrBZ breakdown in adapted sediments

Figure 2. A typical pattern of cross-adaptation to polyhalogenated substrates. Compound in parenthesis indicates substrate used for initial adaptation.

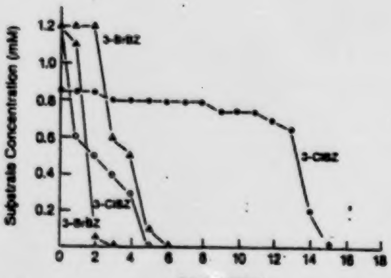

Figure 1

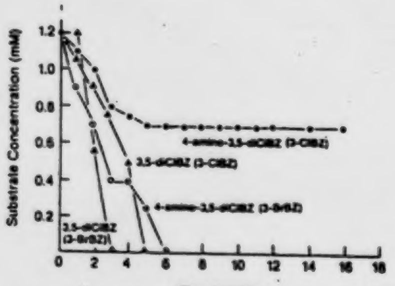

Figure 2

SESSION B

WATER QUALITY RESEARCH

Poster Presentations

BP1

ABSTRACT

The Effects of Agricultural Drainage
on
Sediment and Water Quality Loadings

J.D. Paine, S.A. Peters, W.E. Watt
Department of Civil Engineering,
Queen's University, Kingston, Ontario, K7L 3N6

Impairment of water quality as a result of agricultural activity is desired neither by the farming community nor by recreational users of receiving water bodies. Removal of valuable pesticides, fertilizers and topsoil by surface or subsurface drainage represents an economic loss to agriculture and a potential pollution hazard to receiving waters. In order to evaluate this potential impairment of water quality, there is a need for accurate prediction of chemical contaminant and sediment loadings for different environments, loading conditions, and agricultural management strategies. Such an evaluation is best accomplished by a combination of selective measurement and more general modelling. The model employed should be deterministic and capable of simulating both water quantity and quality at the field level. A few quantity models that meet these criteria are available. However, because of the lack of applicable quality models, a major task in this project is the development of physically-based quality algorithms that can be linked to hydrologic quantity models to accurately predict chemical contaminant and sediment loadings.

An Ontario Ministry of the Environment funded research project (# 152 PL) entitled The Effects of Tile Drainage and Open Ditches on Peak Flows and Dry Weather Flows resulted in completion in 1987 of QTILE, a computer model capable of reproducing water quantity transfer on a tile-drained field and in a small agricultural basin. This model, which was calibrated and verified on two tiled fields in southern Ontario, possesses physically-based parameters which may be easily determined independent of the model itself. Excellent reproduction of flow peaks, volumes and hydrograph shapes was obtained with QTILE.

Assessment of the impacts of tile drainage systems on peak and low flows at the field and small basin levels resulted in the conclusion that tile drainage did not significantly alter the total runoff volume but did change the relative magnitudes of surface and subsurface flows. Because of this alteration in flow paths to receiving streams, processes related to the presence of water, such as soil erosion, nutrient transport and herbicide and insecticide transport and decay mechanisms, might also be significantly changed.

Research was continued in order to link quality algorithms to QTILE to assess the impacts of tile drainage on water quality in receiving streams.

After consideration of the state-of-the-art in quality simulation as it relates to agricultural watersheds, the following research objectives were defined:

1) definition of the processes involved in movement of contaminants through the soil or over the surface and into the tiles or ditches draining agricultural fields,
2) incorporation of an understanding of these processes into a physically-based model capable of simulating water quality changes on a basin scale,
3) collection of field data for calibration and verification of the model,
4) use of the model to evaluate the effects of tile drainage on sediment and water quality loadings, and
5) provision of guidance on the use of the model for evaluation of potential management strategies.

This poster presentation provides an opportunity to describe the progress made in achieving these objectives.

A comprehensive review of existing literature was performed in order to summarize present knowledge of the processes controlling contaminant fates in agricultural systems. Contaminants of interest fall into two groups: nutrients, a grouping which includes common agricultural forms of nitrogen and phosphorus, and pesticides, which includes a wide variety of herbicides and insecticides. Information was collected about sediment transport processes, adsorption-desorption reactions, solution-precipitation reactions, complexation, microbiological reactions, plant uptake, volatilization, photolysis and hydrolysis for these two types of contaminants. These processes operate at different levels of importance depending on the specific contaminant and physical system under study.

The premise of this research is the use of the developed quantity model as a framework for water quality submodels. Hence, preliminary modelling efforts have been directed towards a representation of the physical system which considers chemical transformation of the contaminant of interest as a sink term within a transport mass balance provided by the QTILE quantity model. Later efforts will adapt these water quality algorithms for more sophisticated treatment of specific contaminants.

During development of QTILE, a tiled research field west of Kingston near Napanee, Ontario was instrumented to provide data for model calibration and verification. A collection tank with compound V-notch weir at the tile outlet was installed to monitor tile discharge. Rainfall volumes and rates were determined using a tipping bucket rain gauge and continuous strip chart recorder.

To gather data for the development of water quality algorithms, this monitoring of the Napanee field has been continued and extended. A 24 bottle water quality sampler (ISCO Model 2100), modified to commence sampling once a threshold discharge from the tiled field has been exceeded, has been installed. Following the collection of samples, arrangements have been made for their analysis for contaminant concentrations.

In addition to the Napanee field, a small basin (1.5 km^2) in a corn growing area of the Wilton Creek watershed, has been instrumented for rainfall, runoff and water quality sampling.

BP2

WatQUAS 2.0: AN EXPERT SYSTEM FOR THE WATER QUALITY ASSESSMENT OF ONTARIO RIVERS. Wm. C. Allison, T.E. Unny, University of Waterloo, Waterloo, Ontario, N2L 3G1. L. Logan, Environment Ontario.

The term EXPERT SYSTEM has been applied indiscriminately to many diverse types of computer programs. It has become a "catch-all" phrase for any software that provides the user with more than a numerical response to a problem. The label EXPERT SYSTEM, has evolved into a cliche that is over-used and perhaps not well understood.

"Machine Intelligence" distinguishes true Expert Systems from deterministic computer models. "Machine Intelligence" is the ability of a computer to reach complex inferential solutions to problems. The computer must be capable of searching through many heuristics (rules of thumb) and selecting the appropriate rules that suit each individual situation. A more suitable title for an Expert System which exhibits machine intelligence is an Intelligent Knowledge Based System (IKBS). This name clearly implies that the system contains knowledge that emulates the information stored in the human brain. Searching through a large array of heuristics is the computer equivalent to the human thought process.

The analysis of water quality data is a complex task and it is often difficult for hydrologists to accurately and consistently interpret the results. WatQUAS 2.0 is an Intelligent Knowledge Based System for the assessment of water quality in Ontario Rivers. A comprehensive numerical analysis is conducted on the historical water quality record of a river monitoring site. An expert interpretation of the water quality at the site is completed by utilizing the results from the numerical analysis, a large knowledge base and an inferential engine. Conclusions regarding the origins, seriousness and possible solutions to the water pollution problems are presented by WatQUAS 2.0.

Large quantities of heuristics and domain and expert knowledge are utilized by the Expert System to produce inferential conclusions and recommendations regarding the water quality at the monitoring site. WatQUAS 2.0 possesses a large knowledge base which contains detailed information pertaining to many conventional, organic, and bacteriological pollutants. Approximately 255 different pollutants are contained in the knowledge base. The Canadian Water Quality Guidelines document was an important source of information relating to contaminants in the aquatic environment.

The Ontario Ministry of the Environment "Effluent Monitoring Priority Pollutants List" (EMPPL) was utilized as the basis for determining which contaminants presented a danger to Ontario waters. The chemical hazard rating system developed for the EMPPL is utilized by WatQUAS 2.0 to determine the specific problems presented by each contaminant.

A Data Base Management System is utilized to organize the knowledge block and to permit ease of access to its contents by the users. This feature allows for continual updating and expansion of the knowledge base by the domain expert. RAM requirements for the computer system are also minimized because the Expert System utilizes only the knowledge immediately required.

The knowledge base and heuristics have been greatly expanded and reorganized in this second version of the Expert System. The similar format of the rules for each parameter was recognized in the construction of version 2.0 and rule frames were developed. For most water quality situations only one general rule frame with the specific information being retrieved from the Data Base Management System was used for all parameters

A thorough statistical analysis is conducted by WatQUAS on the water quality data. Both parametric and non-parametric statistical techniques are employed to insure a complete and unbiased analysis.

A new Water Quality Index was developed for WatQUAS 2.0. The new index examines and aggregates the various impacts that each pollutant detected at a site presents to the environment.

WatQUAS 2.0 utilizes the Beale ratio estimator for the calculation of pollutant loads. This is the same method utilized by the Ontario Ministry of the Environment. This ratio estimator permits the Expert System to calculate loads which are more accurate than those calculated by WatQUAS 1.0 and are fully compatible with the requirements of MOE.

The ratio estimator technique also permits WatQUAS 2.0 to identify pollution sources. The quantity of point-source pollution is calculated in the flow stratum representing the base flow. Once identified, the quantity of point-source pollution is subtracted from the total quantity of pollution in non-base flow strata to yield the total non-point source pollution load.

Hypothetical pollutant load reduction is also examined by WatQUAS 2.0 Revised pollutant load estimates are calculated by utilizing a percentage reduction in pollution. This permits water quality managers to examine the effects of control measures and abatement strategies.

WatQUAS 2.0 operates on an IBM compatible micro-computer and is intended for use throughout the Province of Ontario by the Ministry of the Environment. Some computer programming work remains to be completed that will link together the modules that compose WatQUAS 2.0. Future work on the Expert System entails enlargement of the knowledge base, increasing the number of heuristics and expansion of the scope of the WatQUAS system.

BP3

GEOCHEMICAL CHARACTERIZATION, SIZE FRACTIONATION AND BIOAVAILABILITY OF TRACE METAL PARTICULATE ASSOCIATIONS IN THE DON RIVER.

Lesley Warren* and A.P. Zimmerman, Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1.

The total concentration of trace metals found in the receiving waters of the Metropolitan Toronto Area are indicative of a serious contamination problem. It has become increasingly evident that assessment of the environmental impact of metal loadings depends more on knowledge of metal speciation rather than total concentration. Significant fractions of trace metals end up bound to suspended particles with their ultimate fate (burial, resuspension, bioaccumulation *etc.*) connected to the fate of the system's particulate fraction. OMOE has articulated a need to determine the physical and chemical characteristics of suspended particulates in order to assess accurately the impact of metals on aquatic ecosystems. We are in the process of evaluating A). the magnitude of metal transport by suspended sediments in the Don River; B). the geochemical associations of metals with suspended sediments; C). the relationships between particle size, metal load, and geochemical phase; and D). if particle size or geochemical phase have any predictive power for filterfeeding benthic body burdens of metals. Four sites along the Don, progressing from the headwaters to the Bloor Street Viaduct are under investigation. Suspended sediments from these sites were concentrated using continuous flow centrifugation and geochemically characterized using sequential extraction. Periodically at one site, suspended material was size fractionated and geochemical associations were analyzed within particle size classes. The associations of copper, cadmium, zinc and lead with specific geochemical classes were determined using flame atomic absorption. Body burdens of in-situ filter-feeding benthos were analyzed for the same 4 metals. Early results indicate metal levels in suspended material exceed provincial guidelines for dredgeate material (ranges across the 4 sites in ug/g: cadmium 1.9 - 74; copper 18 - 480; zinc 420 - 7000; lead 60 - 2700). Of the 4 metals, cadmium shows the most predictable pattern of binding; it was only associated with the easily exchangeable and iron, manganese oxide fractions. Copper and lead show the most variable patterns of geochemical association with highest levels of copper appearing either in the organic, residual or oxides fractions; while lead levels were highest in the residual or oxides fractions.

BP4

THE INVESTIGATION, EVALUATION, AND RECOMMENDATIONS OF BIOMONITORING ORGANISMS FOR PROCEDURES DEVELOPMENT FOR ENVIRONMENTAL MONITORING. C.A. Jefferson, Curry Jefferson Environmental Services, R.R.# 4, Port Perry, Ontario L0B 1N0.

The problem addressed by this project was the availability of a single, or group of biomonitoring organisms, which could be used in routine contaminant monitoring programs

Programs, requiring discharge monitoring of contaminant levels in biological organisms to determine impact trends with time and space and pinpoint contaminant sources, are expanding. These programs require a common basis for comparison. Consequently organisms proven to provide reliable, consistent and scientifically defensible data, and which are inexpensive tools, must be further developed and utilized.

The specific objectives of the project were to:

- determine if one species of organism was most appropriate for biomonitoring, particularly with respect to a specific discharge type;
- to recommend one species of organism or a combination for either a specific situation, or group of contaminants; to suggest further research needs.

The project involved discussions with researchers; a comprehensive and extensive review of the literature from international, provincial, and federal governments, industry, and educational institutions.

The bulk of the literature pertains to the marine environment with particular respect to molluscs. The freshwater data emphasizes the mollusc. With the exception of work undertaken by the Ontario Ministry of the Environment, no solid database of one particular species or particular contaminant has been developed. Literature on the use of algae, benthic organisms other than bivalves, zooplankton, macrophytes, and fish is not widely available.

The Ontario Ministry of the Environment has the best database of fish/contaminant use.

The project findings suggest that:

- clams be retained and leeches further developed as biomonitoring organisms for relatively short-term studies (1 week to 4 months),

- clams be utilized for certain metals, dioxins, furans, and organochlorine contaminants, while leeches be developed for chlorophenols,

procedures be developed for the use of these two organisms,

- the small yearling yellow perch/spottail shiners continue to be used as long-term (4 months to 1 1/2 years) biomonitors of wider geographic scope.

- clams and leeches can be used singly or in tandem to pinpoint and define the nature and source of the specific contaminant problem.

BP5

THE ONTARIO INLAND LAKES PROGRAM AND MANAGEMENT OF BLUE-GREEN ALGAE: THREE WHOLE LAKE TREATMENTS IN 1988

H. Vandermeulen and K.H. Nicholls
Ontario Ministry of the Environment, Water Resources Branch,
Box 213, Rexdale, Ontario, Canada M9W 5L1

INTRODUCTION

While Ontario's phosphorus control program has achieved measurable reductions in blue-green algal blooms in some inland lakes, there exist many other surface waters for which conventional nutrient loading controls are not practical. One of the objectives of the inland lakes program is to apply other methods to control excessive growths of blue-green algae in eutrophic lakes and reservoirs in Ontario. We are using the "whole lake" approach to accomplish this task. Three different projects were initiated during the summer of 1988, destratification using a Garton style propeller with an upwelling tube at Guelph Lake, hypolimnetic aeration at a small kettle lake in London and a calcium carbonate addition to Puslinch Lake near Cambridge.

METHODS

Destratification - In late September 1988 a 6 m X 6 m raft of welded angle iron with a wire grid deck and ten 230 litre metal drums for floatation was anchored over one of the deepest sections of Guelph Lake. A 4 m long (3.5 m diameter) tube constructed of 2 mm thick polystyrene sheets was attached vertically to the raft 2 m from the lake surface. A metal framework reinforced by guy wires was used to suspend a propeller driven by a submersible electric motor inside the tube, 1 m from the top. The propeller system (Flygt Canada, model 4410 Flomaker) utilized a 3.2 HP motor (600 Volt, three phase) with attached gearbox producing a propeller speed of 32.3 RPM. The propeller was 2.2 m in diameter and used "Banana" style blades which were designed to produce a flow rate of 2.9 m^3 / s. The motor was connected to an electrical supply within the Guelph Lake dam via a 250 m long submersible SOW cable. The destratification system was designed to move bottom water up to the surface of the lake for gas exchange. The system will be removed each winter for storage.

Hypolimnetic Aeration - In early October 1988 a 6.1 m X 2.5 m raft of welded angle iron with a plywood deck and ten 230 litre metal drums for floatation was anchored over the deepest section of a kettle lake in London. A 5.5 m X 1.8 m X 0.6 m deep mixing box constructed of 1.6 mm polystyrene sheets was placed inside the raft and covered by styrofoam SM R10 insulation (10 cm thick on sides, 15 cm thick on bottom). Nine PVC pipes (1.3 cm diameter, 1.8 m long, with 1 mm diameter holes drilled every 2.5 cm) were laid across the bottom of the mixing chamber at 0.5 m intervals. Baffles were placed between the pipes. The pipes were connected to a 0.75 HP oilless compressor (240 volt, three phase, 2 SCFM, 100 PSI) bolted to the deck of the raft. An 8 m long (38 cm diameter) polystyrene inflow tube was attached vertically to one end of the mixing box. A 7 m long (46 cm diameter) polystyrene outflow tube was attached vertically to the other end of the mixing box. The end of the outflow tube was angled to discharge water away from the raft. A metal framework was used to suspend a propeller driven by a submersible electric motor inside the inflow tube, 30 cm from the top. The propeller system (Flygt Canada, model 4400) utilized a 1.5 HP motor (240 Volt, three phase) producing a propeller speed of 1130 RPM. The propeller was 22 cm in diameter and was designed to produce a flow rate of 0.1 m^3 / s. The compressor and propeller motor were connected to a domestic electrical supply (Rotophase converter used to change single phase to three phase) at the lake shore via a 200 m long submersible SOW cable. The propeller pulled water up from the hypolimnion into the mixing box were air supplied via the PVC pipes agitated the water and baffles forced it along a circuitous path to enhance gas exchange. The oxygenated water was returned to the hypolimnion by gravity via the outfow pipe. The system will be removed each winter for storage.

Calcium Carbonate Addition - In early September 1988, powdered limestone (96% $CaCO_3$, 80% of particles <45 um) was brought to Puslinch Lake via tanker truck and blown into the hold of a specially designed trimaran vessel. The vessel had a computerized pumping system to take on lake water and mix it with the powder in the hold. The resulting slurry was deposited on the surface of the lake via spray arms attached to the stern of the vessel covering a width of 18 m. Most areas of the lake deeper than 1 m were sprayed in this manner. A total of 76 metric tonnes of limestone powder was applied within three days.

DISCUSSION

The destratification of a portion of Guelph lake will continue during the summer of 1989. The testing of this system during September 1988 indicated that it does function but the size of the effected area of the lake needs to be determined. It is hoped that destratification will reduce the severity of blue-green algal blooms in the lake as has been reported by other authors. Destratification can prevent an anoxic hypolimnion from forming. Anoxic conditions in bottom waters during the summer months encourages sediment release of nutrients and metal ions, a phenomenon which is correlated with (drives?) the onset of blue-green blooms.

The testing of the hypolimnetic aeration system in the kettle lake has not been completed at the time of writing. The system will be run during the ice free period of 1989 to determine its effect upon the lake and blue-green algae. Hypolimnetic aeration is also expected to prevent sediment release of nutrients, it differs from destratification in that the hypolimnion is preserved. The bottom waters can then be used for maintaining a cold water fishery in a lake were low oxygen levels previously prevented its establishment.

The addition of calcium carbonate to Puslinch Lake should not have a measurable effect upon the lake until the summer of 1989. Slow dissolution of the calcium carbonate during the winter of 1988 will release calcium ions which will bind with phosphorus and lead to a low concentration of this nutrient. Warm water temperatures during the summer of 1989 will then not be coupled with elevated phosphorous levels and a less severe blue-green bloom is expected.

All three of the methods employed are relatively straightforward in theory while the logisics of treatment can be quite involved. Coordination of suppliers and contractors, along with the need to satisfy legal and permit requirements, can lead to long periods of delay due to unexpected problems. Experiments on this scale require time schedules of years rather than months to obtian reliable results. The information and experience obtained from these three projects will be applied to other whole lake treatments in 1989. We hope to be able to test a variety of methods for blue-green algal control and ultimately be in a position to recommend the best treatment for a given lake-bloom situation.

BP6

CHARACTERIZATION OF THE GRAZING FAUNA WITHIN FIVE SOFTWATER LAKES WITH RESPECT TO ACCUMULATIONS OF METAPHYTIC FILAMENTOUS ALGAE.

P.M. Stokes, R.L. France and E.T. Howell. Institute for Environmental Studies and Dept. of Botany, University of Toronto, Toronto, Ontario M5S 1A4.

Introduction

One of the most visually conspicuous of the biological changes related to acidification of surface waters is the development of accumulations of benthic filamentous green algae. These growths are composed mainly of algae from the family Zygnemataceae, and principally from the genera *Mougeotia* and *Zygogonium*. Frequently the growths are metaphytic, appearing as loosely attached clouds up to 2m in diameter, distributed somewhat heterogeneously through the littoral zone. The phenomemon has attracted the attention of the public, for the metaphytic growths appear to have been increasing recently in small softwater recreational lakes on the Canadian Shield. In a 1986 survey conducted for the Ontario Ministry of the Environment (OMOE) of more than 5,000 cottagers in the Parry Sound-Muskoka-Haliburton area of Ontario, the researchers concluded that approximately half of the study lakes had some metaphytic algal growth, and that in 16% of the lakes, the growths were "considerable".

The phenomenon is quite widespread, having been identified in acidifying lakes and streams in southern Sweden, southern Norway, the Adirondacks in New York state, and New Hampshire lakes as well as in south-central Ontario. Experimental acidification has frequently produced similar although not identical effects. Although excessive development of the zygnematacean metaphyton is not restricted to acidifying waters, there is nevertheless a certain consistency in its occurrence, and a number of hypotheses have been put forward to explain this. One of these hypotheses, and the one which provided the major impetus for the present study, is that diminished grazing by herbivores results in less removal of the algae in acidic than in neutral waters, promoting an increase in standing biomass. This says nothing about the productivity of the community, which is the subject of another hypothesis, and of related studies in our group.

Several of the experimental studies on acidification have concluded or suggested that some (unmeasured) decrease in benthic grazing pressure may have contributed to the observed development of algal mats. Certainly a number of zoobenthic organisms which have the potential to consume filamentous algae are adversely affected by acidification, from pH 6.0 and below, but to date the support for the hypothesis has been inferential.

Objectives

The present study set out to:

(a) characterize the algal biomass and potential herbivore fauna in five Haliburton-Muskoka lakes, three of which were known to develop extensive zygnematacean clouds, and

(b) forward an explanation for the algal accumulation and persistence in relation to the abundance of micro- meio- and macro-faunal grazers.

Methods

In the summer of 1987, the five study lakes were selected on the basis of *a priori* knowledge of the presence and absence of filamentous green algal clouds. The lakes have been variously described and studied by the OMOE, and will not be described in detail here. They are: Plastic Lake (pH 5.6, has metaphytic clouds = PL), Gullfeather Lake (pH 5.9, has metaphytic clouds = GF), Bentshoe Lake (pH 5.9, has metaphytic clouds = BS), Crosson Lake (pH 5.7 - 5.0, has so far not developed metaphytic clouds = CL) and Little Clear Lake (pH 6.5, no metaphytic clouds = LC).

Lakes were sampled over the period June - August 1987, with each lake being sampled on two occasions. Metaphyton was sampled quantitatively and the three types of grazers, grouped for convenience as "micro-" (cladocerons, oligochaetes, chironomids etc.), "meio-" (amphipods, gastropods, ephemeropterans etc.) and "macro-" (crayfish, tadpoles and small minnows) were sampled by six different techniques in total. Substrate was taken into account, with major macrophyte communities and sediment type being recorded.

Results

1. Metaphyton biomass. A summary of the biomass of metaphyton is given in Table 1. There was extensive growth of metaphyton in Plastic and Bentshoe Lakes, dominated by *Zygogonium tunetatum*. Gullfeather had less growth, and the clouds were restricted to specific locations, while no such clouds were found in Crosson and Little Clear Lakes. The time of appearance of the metaphyton varied from lake to lake (Table 1).

Table 1. Biomass of metaphytic filamentous green algae in the 0-2 m depth interval in the study lakes. (N=80)

Lake Name[1]	LC	CN	GF	PL	BS
Biomass of metaphyton $g\ m^{-2}$ d.w. (std. err)					
July	0	0	0	0	0.83 (0.20)
August	0	0	NQ	0.97 (0.20)	1.21 (0.29)

note: NQ Growths of metaphyton were observed to occur along 10% of the shoreline however this density of material was considered to low to sample.

[1] See text for full names of lakes.

2. Grazer abundances. There were no overt differences in the abundances of micro- or meio-grazers among the lakes. Notable differences did exist however, with respect to the macrograzers. Crayfish (*Orconectes virilis*) were common in Little Clear Lake, abundant in Crosson Lake (*Cambarus bartoni*, *O. virilis*), but absent or rare in the three lakes characterized by metaphytic algae. Tadpoles (*Rana clamitans*) were also abundant in the non-metaphyton lakes - Little Clear and Crosson, while absent from Gullfeather and Bentshoe Lakes which had *Zygogonium*. Tadpole data for Plastic Lake are inconsistent with the macro-grazer - metaphyton complementarity. Lakes without metaphyton tended to contain greater relative abundances of algivorous fish than those lakes in which algal developments are present. Table 2 summarizes the data for macrograzers. The most notable is Crosson, with a low pH, but with abundant crayfish and no metaphytic clouds.

Table 2. Abundance of macro-grazers in Haliburton-Muskoka lakes. Values in parentheses denote the average standard error of SCUBA determined densities or trap catches between the two census periods. An emprical estimate of crayfish abundance was derived from trap catches with the equationof Capelli (1975) as used by France (1985). Data for fish represent ordinal rankings calculated for cyprinids.

Lake	Crayfish			Tadpoles		Fish
	No. m^{-2}	No./trap[1]	Emp.No. m^{-2}	No. m^{-2}	No./trap	Traps
	(±0.02)	(±0.09	---	(±0.04)	(±0.06)	---
Little Clear	0.18	1.3	0.5	0.36	0.2	3
Crosson	0.3	3.5	1.3	0.33	0.3	4
Gullfeather	0.005	0.03	0.006	0	0	3
Plastic	0	0	0	0.39	0.3	2
Bentshoe	0.01	0.03	0.005	0	0	1

[1] = number of adult males

Conclusion

If alterations in grazing pressure can be implicated as an improtant attribute underlying metaphyton development, our correlative results suggest that it is these algae grazers (particularly crayfish) that will be the most likely candidates. A considerable literature exists providing inferential support for this hypothesis. During 1988, we have surveyed a total of 38 lakes to determine the strength of the relationship observed in 1987.

Acknowledgements

The authors acknowledge financial and logistic support from the OMOE (Research Advisory Committee, Dorset Research Centre) and appreciate scientific discussion with Michael Jackson, Michael Turner and Norman Yan.

BP7

SEDIMENTARY CHRYSOPHYCEAN CYST ASSEMBLAGES AS PALEOINDICATORS IN ACID SENSITIVE LAKES

Mariusz Rybak, Izabela Rybak
ARECO CANADA INC.
180 Elgin St., Ottawa K2P 2K3

and

Kenneth Nicholls
Ontario Ministry of the Environment
Water Resources Branch
125 Resources Road
Rexdale, Ontario

Presented at the 1988 Technology Transfer Conference
Toronto, Ontario
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* The full text of the Report was submitted for publication to PALEOLIMNOLOGY.

ABSTRACT

Relationship between surface sediment cyst assemblages and lake-water characteristics were studied in 50 lakes located in central Ontario. The main purposes of the study were to identify the environmental factors most strongly controlling the distribution of chrysophycean cysts and to develop indices and equations to infer lake water pH from cyst assemblages.

Surface sediment samples were collected from a total of 50 lakes in the Killarney-Perry Sound-Muskoka-Haliburton region of Ontario. The lakes were chosen to represent a wide variety of central Ontario lake-watershed systems (see also Griffiths __et al__., 1988)

Principal components analysis indicates that TDS and associated lakewater pH as well as elements related to trophic status are the most important factors controlling the distribution of chrysophycean cysts. There are significant differences in the relative importance of these factors among the lakes. Generally, the large number of significant correlations suggests that chrysophycean cyst distribution is influenced by one or more of the six environmental factors. Most of the morphotypes seem to be significantly correlated with alkalinity and lakewater pH (21 taxa), while 19 morphotypes appear to be correlated with trophic status of the lake. The results suggests that due to complexity of variables such as pH and trophic status, which can be influenced by several environmental interactions, there could be differences in the level of importance of several environmental factors controlling the distribution of cysts.

Several techniques were used to develop equations for inferring lakewater pH from fossil chrysophycean cyst assemblages. Calibration equations 1 and 2 (Table 1) predicted surface-water pH most adequately and the precision of those equations was the best.

Table 1 Regression equations for inferring pH from fossil chrysophycean cyst assemblages.
All relationship are highly significant ($P<0.0001$)

Equations
1. $pH=7.07+0.193C_{14}-0.153C_{26}-0.031C_{62}-0.327C_{68}+0.176C_{80}-0.119C_{83}-0.107C_{85}-0.280C_{107}-0.331_{AA}$
$R=0.97$ $SE=0.19$ $n=65$
2. $pH=5.836-0.13Acb-0.023Acf0.0148I+0.051Alkf+0.0294Alkb$
$R=0.95$ $SE=0.22$ $n=65$

were C_n - % abundance of the (n) morphotype
Acb - % abundance of Acidobiontic forms
Acf - % abundance of Acidophilous forms
I - % abundance of Indifferent forms
Alkf- % abundance of Alkaliphilous forms
Alkb- % abundance of Alkalibiontic forms

The equations can be recommended for paleoecological studies and are applicable for investigations of a wide pH range of Ontario lake types. It should be noticed that in order to increase the confidence of ecological interpretations based on the derived equations, it was necessary to estimate the relative precisions of cyst-predicted values using data from lakes that were not a part of the calibration lake set (.i.e. the validations lakes); Raven, Maggie, Papineau, Diamond and White). The regression of the pH predicted from equation 1 against the measured has a very high correlation coefficient (R=0.99); SE+).18) and the pH estimated by this equation does not differ significantly (i.e. the intercept and the slopes do differ significantly from zero and one, respectively) from that measured in the validation lakes. Although the relationship between the predicted and measured pH in the validation lakes have a very high R values based on other derived equations.

The study also provides a descriptive analysis of the "fossil" chrysophycean cyst flora from Ontario lakes. The descriptions include representative SEM micrographs and detailed characterization of each morphotype in consideration of the morphological variation observed among specimens of the same morphotype. Special attention has been paid to the anatomy of the collar complex and to the nature of the cyst surface ornamentation. One hundred thirty seven morphotypes are described, most of them for the first time.

The present study as well as previous investigations (Rybak, 1986; Rybak, 1987; Rybak *et al.*, 1987) show a great potential for using chrysophycean cysts as paleoindicators.

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BP8

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Great Lakes Institute, University of Windsor, Windsor, Ontario. N9B 3P4

Three species of forage fish : Labidesthes sicculus (Brook Silverside), Notropis hudsonius (Spottail Shiner), and Pimephales notatus (Bluntnose Minnow) were collected from three sites along the St. Clair River system during July, August, and September 1987. The fish were caught in nearshore waters using a 0.6 cm mesh bagseine. They were measured (total length) and immediately wrapped in hexane-rinsed aluminum foil. Samples were kept frozen at -20 degrees Celsius until they were analyzed.

The three species are morphologically distinct and it is these physical differences that separate them ecologically (Keast 1966). L. sicculus is primarily a surface feeding species whose beak-like snout and dorso-terminal mouth are designed to seize prey at the surface. P. notatus possesses a ventro-terminal mouth designed for benthic feeding. N. hudsonius has a terminal mouth and is more omnivorous than the other two species. Therefore, there was an integration of the foodweb with L. sicculus tracking allochthonous surface inputs, N. hudsonius pelagic food resources, and P. notatus benthic foodwebs. There would have been some overlap in resource utilization but the specialized feeding adaptations of L. sicculus and P. notatus would have limited this resource sharing.

Whole fish were analyzed according to the protocol developed by the Canadian Wildlife Service (1982). The samples were injected into a GC-ECD. Pentachlorobenzene (log K_{ow}=5.0), hexachlorobenzene (log K_{ow}=5.5), and octachlorostyrene (log K_{ow}=6.2) were examined in detail due to their presence in all of the samples and because of their range in K_{ow}s (Oliver 1987).

The levels observed for all the contaminants were higher at Site 1 due to that site's proximity to the source of contaminants in Sarnia's Chemical Valley. No significant differences were observed in contaminant levels between Sites 2 and 3 (one-way Anova, $p<0.5$) which were located in the Walpole Island marshes. At all sites, *P. notatus* had the highest mean contaminant levels (levels are $ugkg^{-1}$, Site 1; QCB=6.73,HCB=62.84,OCS=140.67 / Sites 2+3; QCB=1.23,HCB=12.34,OCS=32.61) followed by *N. hudsonius* (Site 1; 5.77,40.21,73.27 / Sites 2+3; 0.68,8.19,22.75), and then *L. sicculus* (Site 1; 2.24,10.55,4.19 / Sites 2+3; 0.77,4.07,2.58). As the log Kow of the compounds increased these interspecific differences became more significant. As expected there was an increase in contaminant level with increasing log K_{ow}.

There were no significant interspecific differences in size or lipid content between species (one-way Anova,$p<0.05$). Lipid normalized HCB and OCS levels showed significant interspecific differences (one-way Anova,$p<0.05$ and $p<0.01$ respectively) but QCB did not (one-way Anova,$p<0.1$). Samples containing gutted *P. notatus* were found to have insignificantly different contaminant burdens than comparative samples consisting of whole fish (one-way Anova, $p<0.5$). Therefore, gut contents did not bias contaminant measurements.

The lipid normalized interspecific differences in contaminant burdens indicated that bioaccumulation was a major factor regulating contaminant levels in forage fish. This was especially true for the higher K_{ow} compounds, HCB and OCS. Bioconcentration, however, may have been more important for the lower K_{ow} compound, QCB. This would have accounted for the smaller interspecific difference that was seen for this compound in that QCB exposure would have been more homogeneous among the three species. Due to their relatively high K_{ow} and low aqueous solubility HCB and particularly OCS would be expected to partition more readily into sediment. When feeding, P. notatus is in continual contact with the substrate and may ingest a substantial amount of sediment with its food items. This was perhaps the best explanation as to why this species had consistently higher contaminant burdens particularly with respect to the higher K_{ow} compounds. N. hudsonius, a facultative omnivore, generally had higher contaminant burdens than L. sicculus but lower levels than P. notatus. This might have been due to its selection of a wide variety of food items which varied in their degree of contamination. The levels of contaminants in L. sicculus remained much lower than in the other two species because a large proportion of its diet consisted of terrestrial insects with lower HCB and OCS levels.

Ratios of contaminants are useful in that they yield information on foodweb interactions. Some organochlorine contaminants with high K_{ow}s will partition more readily to benthic systems whereas others with greater aqueous solubility and lower K_{ow} will remain in the pelagic system. The proportion of one type of compound to another will give an indication of where an organism is primarily feeding. Using this assumption, organochlorine compounds may be thought of as ecological markers highlighting foodweb interactions (Flint 1988). For *L. sicculus* it was observed that the ratio of lower to higher K_{OW} compounds was greater than the ratios observed for the other two species (Ratio HCB:OCS; *P. notatus*=0.4, *N. hudsonius*=0.4, *L. sicculus*=1.9). If habitat utilization and therefore food selection was important to contaminant uptake then this would be expected. *L. sicculus* was not exposed to OCS contaminated sediment or to foodwebs associated with benthic habitats due to its surface feeding behaviour.

The interspecific differences that were observed indicated that habitat partitioning was a major factor regulating contaminant levels in these forage fish species. Although chemical and physiological parameters may determine which contaminants have the potential to bioaccumulate, it is the regulation of exposure through ecological processes that will determine the degree to which that potential is realized.

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BP9

THE ISOTOPIC COMPOSITION OF UPLAND FOREST SOIL SULPHATE.

D.R. Van Stempvoort, P. Fritz, and E.J. Reardon

Dept. of Earth Sciences, University of Waterloo
Waterloo, Ontario N2L 1M5

The environmental impact of acid rain sulphate has become an issue of both national and international concern. The Plastic Lake watershed (Dorset, Ontario) is an acid rain-affected site that has been the subject of an intense geochemical investigation by Environment Ontario over the past 8 years. The sulphate balance in upland forest soil at this site can be modelled simply as a input of sulphate from the atmosphere, and output of sulphate to groundwater, neglecting geochemical processes and transformations within the soil. However, this broad approach is subject to large errors, such as large uncertainty in the rate of dry deposition of atmospheric SO_2 and particulate sulphate. Similarly, some throughfall sulphate may have leached from leaves of plants that incorporated the sulphate by roots from soil. This possibility creates more uncertainty in the calculation of input atmospheric sulphate.

Furthermore, a number of previous biogeochemical studies indicate that soil sulphate is not "conservative", but is incorporated by plants and microbes, and also is a product of microbial mineralization of soil organic compounds. Some of the soil water sulphate may be adsorbed and retained indefinitely by iron and aluminum oxyhydroxides. It remains unclear just how these processes affect the impact of sulphate on forest ecosystems.

In this investigation, the detailed dynamics of sulphate in the upland forest ecosystem at Plastic Lake (1986-88) have been documented, in part, by stable isotope analyses of both sulphur and oxygen in sulphate. These isotope ratios provide direct tracers of the fate of atmospheric sulphate in the soil. In this ecosystem, there is very little variation in the average sulphur isotope ratios ($\delta^{34}S$ o/oo (CDT)) of sulphate in various reservoirs (rainfall, +3.8; throughfall, +4.1; stemflow, B horizon leachate, +4.5), or fractions of soil organic sulphur (leaves, +3.9; litter, +3.7; humus, +3.5). This lack of variation reflects the dominance of atmosphere-derived sulphur in this ecosystem, and the nonimportance of sulphur isotope fractionating processes.

Furthermore, isotope measurements indicate that most (70-100 %) of the sulphate in throughfall is derived from the atmosphere: Throughfall sulphate (avg. $\delta^{18}O$ = +10.9 (SMOW)) and stemflow sulphate (avg. $\delta^{18}O$ = +9.8) have slightly lighter oxygen than the precipitation sulphate (avg. $\delta^{18}O$ = +11.4). Since throughfall makes up around 90-95 % of upland forest soil infiltration, it is clear that leaching of soil-derived sulphate from aboveground vegetation is not a major component of the sulphur cycle.

Of great significance, dissolved and water soluble sulphate in the upland forest soil has a distinct oxygen isotope composition (avg. $\delta^{18}O$ = +5.5) relative to local precipitation sulphate ($\delta^{18}O$ = +11.4). This must be due to chemical or biological reactions in the soil. Adsorption of sulphate by aluminum and iron oxyhydroxides is a very important process in many acidic soils, including the B horizon (sandy till) of the upland Podzols in the Plastic Lake watershed. However, field data and our ongoing lab studies indicate that this process has a minor effect on the isotope composition of sulphate in soils. In some of our tests, dissolved sulphate is enriched in ^{18}O relative to sulphate adsorbed by iron oxyhydroxides, opposite in direction to that expected if adsorption was responsible for the soil sulphate oxygen shift (dissolved sulphate depleted in ^{18}O). In fact, the soil sulphate oxygen isotope shift is already detectable in leachate samples from the uppermost litter-humus zone (avg. $\delta^{18}O$ = +5.8), where sulphate adsorption is minimal. Clearly this indicates that soil adsorption is not the cause of the observed oxygen shift.

In light of the above evidence, plant and microbe mediated processes, focussed in the litter-humus zone, are likely responsible for the soil sulphate oxygen isotope shift. Plant and microbe bioassimilation of sulphate is a ubiquitous process in soils. Reduced organic sulphur compounds are recycled by soil microbes, and in aerobic upland soils mainly mineralized to sulphate containing new soil-derived oxygen atoms. Our ongoing lab experiments indicate that the approximate oxygen isotope composition of secondary mineralized sulphate at Plastic Lake is roughly +0.5 o/oo ($\delta^{18}O$). Based on this preliminary data it appears that soil sulphate is approximately composed of 55% secondary sulphate, and 45% residual atmospheric sulphate.

This evidence suggests that soil biological processes exert a partial control on the effect of acid rain sulphate, but clearly these biological processes do not completely control the release of acid rain sulphate to subsoil horizons. Future studies of the impact of acid rain must address these soil biological processes in more detail, and stable isotope analyses appear to be a valuable tool for such work.

BP10

RECENT TRENDS AND HISTORICAL CHANGES IN WATER QUALITY OF LAKE MUSKOKA

PROJECT 381 C

Mariusz Rybak, Izabela Rybak
ARECO CANADA INC.
180 Elgin St., Ottawa, Canada K2P 2K3

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ABSTRACT

Lake Muskoka, the largest lake in the district of Muskoka, provides a representative example of lake ecosystem under anthropogenic stress. The lake is located in an acid sensitive region with limited neutralizing capacity. It is exposed to acid precipitation as well as acidic run-off water from the surrounding areas. The stability of the ecosystem is also effected by eutrophication and contamination processes. The lake has an area of localized nutrient enrichment which causes periodic algal blooms (Gravenhurst Bay) with all the negative environmental changes related to it.

The primary objective of the study was to analyze and document long-term water quality changes in the lake with emphasis on heavy metal contamination and eutrophication problems.

The approach to this research was based on the application of several paleoecological techniques. Detailed analysis of metals deposited in lake sediments was used to estimate the extent of lake contamination induced by erosional inputs and atmospheric deposition. Analysis of major oxides, supported by fossil pigment analysis provided an assessment of the degree of lake eutrophication as well as exchange of cations between the sediment and the water column (induced by changes in hypolimnetic

oxygen regime). Fossil pigment analyses (as primary) was applied to reconstruct the history of the lake's productivity and changes in the past and present trophic status. The history of blue-green algal development in relation to the eutrophication process was documented using detailed quantitative fossil pigments analysis (myxoxanthophyll and oscillaxanthin). Recent events were accurately dated using the Lead-210 radionuclide method.

RESULTS

Trace metal contamination

In this study, 88 (eighty eight) sediment samples were analyzed for the trace metals Co, Cd, Ni, Mn, Cu, Zn, Zr, Hg, and Pb. The data indicate a significant spatial variation of heavy metal contamination among 24 studied stations. The highest concentration of metals was recorded in the core sediment samples originating from Cooper Point. Many of the measurments exceeded the Ontario MOE guidelines. Most of the analyzed elements in sample core samples studied elements showed a systematic change in concentration with depth suggesting, anthropogeniclly related trace metal accumulation.

Trophic Status

Stratigraphic shifts in the concentration of chlorophyll and carotenoids have often been interpreted as evidence of changes in primary production and trophic status. Most of the study sites in the lake showed similar levels of chlorophyll derivatives, total carotenoids and blue-green algal pigment concentration with the exception of Gravenhurst and Muskoka Bays. In both cores from those two locations, the concentration of pigments showed successive increases upwards to the sediment surface. Only the most upper strata indicated a lower level of pigment concentration. This may suggest significant changes in the trophic status and level of primary production resulting from the phosphorus removal operation initiated in 1971 at the town of Gravenhurst.

BP11

Metal Contamination of Wetland Foodchains in the Bay of Quinte, Ontario

A. Crowder*, W. Dushenko and J. Greig, Dept. of Biology, Queen's University, Kingston, Ontario.

In September, 1987, the Lake Simcoe Regional and Metropolitan Toronto and Regional Conservation Authorities began research on the survival characteristics of fecal and water quality indicator bacteria in rural watersheds. Six locations within the confines of the LSRCA and MTRCA Rural Beaches Study areas were selected for examination. These sampling site locations included: 3 sites in Pefferlaw Creek (LSRCA); 1 site in the East Humber River; and 2 sites in Centreville Creek (MTRCA). The bacterial parameters studied during the first year of this 2 1/2 year project included: pure cultures of *Escherichia coli* and *Pseudomonas aeruginosa*, and fecal coliforms and *Pseudomonas aeruginosa* in mixed cultures containing total heterotrophic bacteria.

The water column survival of these bacteria has been assessed at each of the 6 sites under 4 seasonal conditions: fall (September/October); winter (January/February); spring (April/May); and Summer (June/July). In addition to the water column work, sediment survival runs of *E. coli* were completed at each of the sites under the above same seasonal conditions, making a total of 100 runs to date. Water column temperature and chemical parameters such as dissolved carbon; total phosphorous and nitrogen; nitrates; and ammonia were monitored during the runs to determine their effect on bacterial survival. Bed sediment nutrient levels and particle sizes were also analyzed. Invitro (Laboratory) survival experiments were conducted to measure the effect of specific parameters, i.e. water temperature and nutrient content on bacterial survival under controlled conditions.

Water column bacterial die-off rates in the 3 watersheds ranged from 0.1 to 0.35 Log Units/Day for *E. Coli*; 0.02 to 0.4 Log Units/Day for fecal coliforms; 0.03-0.28 Log Units/day for *P. aeruginosa*; and 0.01 to 0.25 Log Units/day for P. aeruginosa (Mixed culture). The rates exhibited were considerably slower than those reported for urban rivers and streams (0.5 to 1.0 Log Units/day) (Seyfried, Harris and Young; 1988 unpublished).

Some seasonal effect on the *E. coli* and fecal coliform rates was observed with more rapid die-off occurring during the summer (Pefferlaw Creek) and spring (East Humber River and Centreville Creek). Results of the in-laboratory experiments confirm that more rapid die-off of EC and FC occurs under warmer temporal conditions. Rates for *P. aeruginosa* and *P. aeruginosa* (mixed culture) tended to be slower during warmer weather in Pefferlaw Creek however, this trend was not as evident in the 2 MTRCA watersheds.

E. coli die-off in bed sediments was less rapid than in the water column (0.01 to 0.1 Log Units/day). Somewhat faster rates were noted during the winter in Pefferlaw Creek and during the spring in the East Humber River and in Centreville Creek. Apparent regrowth of *E. coli* in the sediments was observed during the fall and summer survival runs in all 3 watersheds. This phenomena has been observed by previous investigators and is thought to result from the use of autoclaved sediments in conjunction with the warmer seasonal temperatures.

Statistical comparisons of the die-off rates between sites (during an individual survival run) were performed by regression analysis. The results of these analyses show that bacterial survival in both the water column and bed sediments can vary within a watershed, and is thus site specific.

The in-situ relationship between water temperature, nutrient concentration and bacterial survival has not been fully established at this time. It would appear though, that within the EC/FC group, survival is somewhat enhanced when both water temperature and nutrient levels are in positive correlation i.e. high temperature/high nutrients or low temperature/low nutrients. This same trend was also exhibited during the invitro survival experiments. In-laboratory experiments to assess the effect of temperature and nutrient levels on *P. aerugimosa* survival have not been completed yet.

2.0 METHODS

The methodology for conducting survival runs incorporated the use of membrane diffusion chambers developed by G. McFeters of the Montana State University. (McFeters and Stuart, 1972; 1981). Further modifications to the current method, including the design of the sediment and water column chamber holders, were made by G. Palmateer of the Ministry of the Environment, London, Ontario.

Chambers for water column runs were prepared by inoculating bacterial cultures, diluted 10 fold in sterile site water, into sterile diffusion chambers. Time zero control samples were taken and analyzed to determine the initial bacterial concentration. The chambers were then transported in containers of chilled (4°C) site water to the test locations. Triplicate chambers of the bacterial suspensions were placed into anchored holders at each site.

Sampling of the chambers was conducted over a two week period commencing with daily sampling during the first week of the run and 2 samplings during week 2.

Samples were transported to the Laboratory, on ice, where they were analyzed within 24 hours by membrane filtration (MF) using the following selective media: M-TEC for the determination of fecal coliforms and *E. coli* (Dufour, 1981), M-PA for the determination of *P. aeruginosa* (Standard Methods, 1985) and MSPC I for the determination of total heterotrophic bacteria. (Standard Methods, 1985).

Sediment survival chambers were prepared by inoculating 5 mls of an *E. coli* culture, diluted 10 fold in sterile site water, into chambers containing 50 grams of sterilized site sediment. Nine chambers were prepared per site, of these, 8 were transported to the field in chilled site water and the remaining chamber analyzed as a time zero control.

Sampling of the sediment chambers was accomplished by removing 1 chamber from each site for analysis at 24 and 72 hours and once per week thereafter for 6 weeks. Sediment chamber samples were analyzed by MF or MPN technique depending on the bacteria level. EC Broth (Difico) + Methyl Umbelliferyl-B-D-Glucoronide (MUG) (Feng and Hartman, 1982) was as the enrichment medium used in the MPN procedure.

Invitro survival experiments were conducted using 10 gallon aquariums filled with water from Pefferlaw Creek. The tanks were placed in 10°C and 20°C incubators to achieve and maintain the 2 desired temperatures. The preparation methods for water column survival chambers of *E. coli* and *P. aeruginosa* were identical to those used for the inside survival runs. Triplicate chambers of the bacteria were prepared for testing at both 10°C and 20°C and were emersed in the tanks immediately. Sampling of the invitro chambers was conducted over a 7-day period and the samples were analyzed in the same manner as were the inside samples.

Calculation of the bacterial die-off rates for both inside and invitro survival runs was performed by regression analysis of bacterial concentration with time.

BP12

Development of an Acute and Chronic Sediment Bioassay Protocol Using Larval Mayflies and Juvenile Fathead Minnows. **Gail Krantzberg[1] and Richard Pope[2], [1]Water Resources Branch, Ontario Ministry of the Environment, Toronto, Ontario M4V 1P5, [2]Tarandus Associates Inc., 21 Greystone Crescent, Brampton, Ontario L6G 2B2.**

OVERVIEW OF SEDIMENT BIOASSAY

Sediment bioassays measure the effects of contaminated sediments on the biota. Sediment elutriates have been prepared as liquid phase matrices, principly to assess the impacts of dredging activities on water column organisms (1,2). For example, one toxicity test exposes Daphnia to an elutriate (3). Pore waters have been considered as an alternate liquid phase to examine the effects of contaminated sediments on the burrowing infauna and to identify the route of exposure of different organisms to different pollutants (4).

By far the most frequently described approach is solid phase testing with either benthic or water column organisms (5,6). For the purpose of evaluating the impacts of inplace pollutants on the biota, as opposed to the consequences arising from dredging operations, the focus of this study was on the solid phase bioassay.

The principle objective of this study was to contribute to the development of a methodology for assessing the chronic and acute toxicity of sediments to biota. This included an examination of the effects of bioassay assembly and sediment manipulation techniques to the response of the test organisms, and the sensitivity of growth as a chronic endpoint.

Experiment 1: To determine the efforts of settling time, following the addition of sediments and water to the bioassay container, on toxicity to mayflies.

It is reasonable to expect that the exposure of an organism to contaminants will vary with the state to which the sediment-water system is in equilibrium. We therefore examined whether an organism's response varied with the length of settling time of the bioassay assembly proceeding the introduction of the organism. The duration of exposure required for the response of organisms in test sediments to differ significantly from the controls was also not known. As a result, the experiment was designed so that half of the replicates could be harvested at day 10 and half could be harvested at day 21.

2L widemouth glass jars were filled to a depth of 3 cm with sediment (surface area = 100 cm²) and water was gently added. Organisms were introduced at 3 time intervals; 5 hours settling plus 1 hour of aeration, 1 day settling plus 1 hour and 5 days settling plus 1 hour aeration. At each time interval, either 8 mayflies (c.a. 25 mg/ individual wet weight) or c.a. 1.5 gm oligochaetes wet weight (c.a. 150 individuals) were added to the chambers. Each treatment had 4 replicates. Water and sediment samples were collected as animals were added and when replicate containers were harvested (time = 10d or 21d).

Analysis of the growth response of Hexagenia suggested that biomass changes were influenced both by sediment type and by the duration of the period of equilibration (Table 1). Growth in both test sediments was greatest when the mayflies were added 5 days after chamber assembly, followed by a 1 day equilibration period. Growth was poorest when organisms were added 6 hours following assembly (1 hour after aeration). Growth inhibition more pronounced by day 21, as compared to day 10.

Experiment 2: To determine the effects of settling time on toxicity to fathead minnows at 2 different densities.

Fathead minnows weighing c.a. 0.5 gm per individual were added to each bioassay chamber at a rate of 10 or 15 individuals per replicate. Four replicates of each treatment were harvested after 10 or 21 days exposure.

In accordance with the biomass changes noted for mayflies, growth inhibition was least when the fathead minnows were added 5 days after chamber assembly. There appeared to be no notable difference between the 6 hr. (5 hr. settling plus 1 hr. aeration) and 1 day equilibration periods with respect to biomass changes, and the effects of fish density were variable. Growth inhibition was greater with 15 as compared to 10 fish in some, but not all cases, and density apparently exerted no influence on biomass changes in the controls. This last finding is of interest, since it may indicate that the stress of possible overcrowding was exacerbated by the contaminated sediments. By day 21, all fish had decreased in weight. Minnows from the test sediments lost more weight than did the controls.

Experiment 3: To compare the toxicity of intact sediment cores to homogenized sediments for mayfly nymphs and fathead minnows.

Current methods for assembly of sediment bioassays often involve sieving and homogenizing the sediment. This effectively exposes the organisms to a uniform dose of contaminants that is in reality a mean dose of the heterogeniously distributed contaminants. In some cases, the extensive aeration of the sediment also results in a transformation of chemical species to forms that are of greater or lesser bioavailability. We examined the question of sediment homogenization by using diver-collected cores. The cores used were acrylic tubes of comparable surface area to the 2L glass jars. Organisms were introduced into the cores and into homogenized sediments from the same site as those where cores were collected. Eight Hexagenia nymphs (c.a. 40 mg/individual net weight) or 10 juvenile fathead minnows (c.a. 400 mg/individual net weight) were the test organisms. Mortality and biomass changes over three weeks were the endpoints examined. pH and dissolved oxygen were monitored in all chambers.

In Site A, intact sediments resulted in higher mortality and poorer growth than homogenized sediments for mayfly nymphs, but did not significantly influence mortality or growth in fathead minnows. Intact sediments from Site B resulted in better growth for mayfly nymphs than homogenized sediment. Mortality was <10% in both treatments. Homogenization resulted in substantial mortality for fathead minnows (87% vs 20% in intact cores). In Site C (sandy sediment), homogenization resulted in higher mortality than in the intact cores for Hexagenia. This was most likely caused by the elimination of the surface layer of fine-grained material (present in intact cores) and therefore, the elimination of suitable substrate for burial and feeding. Homogenization did not effect growth of fathead minnows, and may have ameliorated toxicity as measured by mortality.

TABLE 3: Effect of Settling Time on Growth of *Hexagenia limbata*
Values in parentheses are standard deviations

MEASUREMENT	SETTLING TIME							
	5h	24h	120h	5h	24h	120h	5h	24h
	Toronto STP			Rice Lake			Control	
Percent Biomass Change (Day 10)	-3 (0.9)	-2 (0.1)	25 (7)	17 (2)	30 (6)	25 (10)	103 (8)	113 (3)
Percent Mortality (Day 10)	4 (5)	6 (7)	12 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Percent Biomass Change (Day 21)	4 (9)	18 (10)	69 (3)	42 (9)	77 (25)	129 (50)	163 (10)	159 (15)
Percent Mortality (Day 21)	7 (9)	12 (10)	12 (10)	8 (11)	8 (11)	0 (0)	0 (0)	0 (0)

As a result of these preliminary experiments, we recommend further detailed examination of bioassay design and chronic endpoints, including bioaccumulation, in order to determine the significance of inplace pollutants.

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BP13

PULSE EXPOSING RAINBOW TROUT EGGS TO POTASSIUM THIOCYANATE: EFFECT OF WATER HARDENING

Sherrene D.Kevan and Dr. D.G. Dixon

Department of Biology, University of Waterloo, Waterloo,Ont. N2L 3G1.

INTRODUCTION

Thiocyanate (SCN-) is a pollutant of mining effluents when cyanidation is used to leach precious metal from ores. Although SCN- effluents are released continuously, short-term or pulse exposures of aquatic organisms occur because of accidental spills, batch release of effluent, or organisms enter a mixing zone.

The impact of SCN- pulse exposure on eggs of rainbow trout during their water hardening has not been investigated before.

During water hardening, the chorion or fertilization membrane of the egg becomes impervious to water penetrating into the egg. Before water hardening, for about one hour after the egg has been deposited, the egg rapidly absorbs water from its surroundings. Thus, one would expect water soluble and toxic ions, such as SCN- to have a more pronounced effect in killing eggs before they water harden than afterwards. The aim of this study was to examine that problem.

MATERIALS AND METHODS

Rainbow trout eggs were obtained from Rainbow Springs Trout Farm (Thamesford, Ont.). Two bioassays were conducted using 4 replicates, each containing 100 to 150 eggs and 7 KSCN concentrations of 0 (control), 90, 180, 360, 720, 1440, 2880 mg/l. The eggs were split into two groups. The first group was dry-fertilized and then exposed to KSCN for 3 hours during water hardening. The second group was fertilized and allowed to water harden in clean water for 3 hours before the 3 hour exposure to KSCN. Once the eggs were treated, they were randomly assigned to compartments within 8 trays of a Flex-a-lite incubator where they were allowed to develope up to hatch.

During incubation, temperatures and flow rates of the water were monitored. Fertility estimates were made on subsamples of 50 eggs preserved in Stockard's solution 14h postfertilization. Eggs in the incubator were checked regularly and dead eggs removed weekly. Egg mortality and developmental abnormalities after hatch were recorded. Chi square analysis was carried out by Epistat* program. The LC50 and 95% confidence limits were determined by Trimmed Spearman-Karber analysis.

RESULTS

The estimated LC50 for the treatment before hardening was 2018.749 (95% confidence limits = 1898.042 to 2147.132) and for after hardening it was 1530.268 (95% confidence limits = 1408.536 to 1662.520). These are significantly different.

Chi square analysis showed that there are no clearly significant differences in mortality. Before treatment mortalities ranged from 16.8 to 26.0% but after treatment mortalities ranged from 19.3 to 23.5% for concentrations of 90 to 720 mg/l KSCN. At 1440 mg/l there was a highly significant difference ($p < 10^{-9}$). There was no significant difference in mortality (78.8% before and 72.2% after) among treatments at the highest concentration (2880 mg/l). Clearly, mortalities at 2880 mg/l are significantly higher than at other concentrations for both before and after hardening, but at 1440 mg/l, only the eggs treated after hardening showed significant difference from those treated with lower concentrations.

Percent deformities were greatest at 1440 and 2880 mg/l in both treatments (ca. 2-6 % at lower concentrations, but between 7.1 % and 9.6 % at the higher concentrations). Fertilization rates were reduced in eggs treated both before and after hardening at 2880 mg/l (by 54% and 40% respectively) versus at the lower concentrations.

DISCUSSION

Exposure to concentrations of KSCN had a more adverse effect on trout eggs after water hardening for 3 hours than on eggs that had not water-hardened. This result is contrary to the original hypothesis, but is consistent with the results of other investigators. Perhaps the age and metabolic activity of eggs after hardening makes them more susceptible to poisoning. Fertilization was affected at the highest concentrations in both treatments but was more pronounced in the before hardening treatment. Eggs appear to be more sensitve directly after fertilization. Deformities which were observed in both treatments, and at all concentrations, were not significantly different. The action of the SCN- ion is affecting the metabolic process of the egg in before and after stages of hardening. Overall, eggs of rainbow trout are most sensititive to reductions in fertilization, deformities in development, and mortality after water hardening has occurred than before.

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A1 **Science and Policy: PhotoChemical Oxidants and Acid Bearing Species** K. L. Demerjian, Atmospheric Science Research Center, State University of New York, Albany, New York, U.S.A.

A2 **Relationship Between Forest Decline and Root Health in Ontario Sugar Maple** C. Adams, M. Egyed and T. Hutchinson*, Dept. of Botany, University of Toronto, Toronto, Ontario.

A3 **A Numerical Decline Index Rating System to Monitor Changes in Tree Condition of Hardwood Forest Species** D. McLaughlin*, W. McIlveen, W. Gizyn, D. Corrigan, R. Pearson and R. Arnup, Air Resources Branch, Environment Ontario

A4 **Investigation of Short-term Mutagenicity and Chemical Composition of Organic Solvent Extractable Fraction of Coke Oven Emission** A.J. Horton*, N. Belson, K. Shaw and G.H. Thomas, Ontario Research Foundation, Clarkson, Ontario

A5 **Quantitative Measurements of the Genetic Effects of Inhaled Carcinogens in Pulmonary Fibroblasts are Now Possible** J.A. Heddle*, A. Bouch and J.D. Gingerich, Dept. of Biology, York University, Downsview, Ontario

A6 **Sensitivity of Asthmatic Children to Air Pollution;** D. Pengelly* and C. Goldsmith, McMaster University, Hamilton, Ontario

A7 **Hazardous Contaminants in Ontario: Environmental Fate and Human Exposure** D. Mackay* and S. Paterson, Institute for Environmental Studies, University of Toronto, Toronto, Ontario

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A8 **Verification of the Cloud and Wet Deposition Fields of a MesoScale Model of Long-Range Transport of Air Pollutants** H.R. Cho*, S.T. Soong and J.V. Iribarne, Department of Physics, University of Toronto, Toronto, Ontario

A9 **Eulerian Model Evaluation** M. Alvo, Department of Mathematics, University of Ottawa, Ottawa, Ontario

A10 **Scale Model Studies and Development of Prediction Procedures for Heavy Gas Dispersion in Complex Terrain 1988** P. A. Irwin*, M.C. Murphy and K. C. Heidorn, Rowan Williams Davies and Irwin Inc., Guelph, Ontario

A11 **An Investigation of Wind Generated Particle Transport Rates within a Turbulent Boundary-Layer** A.D. Ciccone*, J.G. Kawall and J.F. Keffer, Department of Mechanical Engineering, University of Toronto, Toronto, Ontario

A12 **Incineration of Wastes** K. Davies, Environmental Protection Office, City of Toronto, Toronto, Ontario

A13 **Detectability of Step Trends in the Rate of Atmospheric Sulphate Deposition** E.A. McBean*, M.G. Kompter and G.J. Farquhar, Department of Civil Engineering, University of Waterloo, Waterloo, Ontario

A14 **Incinerator and Steel Plant Contributions to Air Particulates as Determined by Size-Specific Receptor Modelling** A.C. Chan*, Z-J. Kang and R.E. Jervis, Dept. of Chemical Engineering, University of Toronto, Toronto, Ontario

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A15 A Study on the Sources of Acid Precipitation in Ontario, Canada P.K. Hopke* and Y. Zeng, Department of Civil Engineering, University of Illinois, Urbana, Illinois, U.S.A.

A16 Advanced Techniques for Mobile Monitoring of Trace Organics in Air G.B. De Brou*, E. Singer, M.A. Sage, R. W. Bell, R.E. Chapman and D.J. Ogner, Air Resources Branch, Environment Ontario

A17 Atmospheric Trace Gas Measurements Using a Tunable Diode Laser Absorption Spectrometer D.R. Hastie* and H.I. Schiff, Department of Chemistry, York University, Downsview, Ontario

A18 Biomedical Waste Incineration Testing Program V. Ozvacic*, G. Wong, G. Marson, R. Clement, D. Rokosh, S. Suter, G. Horsnell, J.C. Hipfner, S. Burns and H. Corinthios, Environment Ontario

A19 A Study of High Temperature Photochemical Kinetics of Sulphur Dioxide and Nitrogen Oxides For a Flue Gas Treatment Process J. Hunt*, P. Fellin, K.A. Brice, D. Ernst, D. Glendenning and R. Caton, Concord Scientific, Toronto, and C. Fung and K. Smith, Environment Ontario

A20 Modelling the Photochemical Decomposition of Chlorinated Phenols by Sunlight N.J. Bunce* and J.S. Nakai, Dept. of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario

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AP1 **Stochastic Modelling of Dispersion from Single Elevated Sources** E. Robertson and P. J. Barry, Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario

AP2 **Feasibility Study for Assessing and Modelling Microclimatic Conditions on the Fonthill Kame (Phase 1)** T.B. Shaw, Brock University, St. Catharines, Ontario

AP3 **Critical Evaluation of Atmospheric Pollutant Parameterization from Satellite Imagery** N.T. O'Neill, A. Royer and L. Hubert, Universite de Sherbrooke, Sherbrooke, Quebec, and J. Miller and J. Freemantle, CRESS, York University, Downsview', ',Ontario, and G. Austin and A. Davis, McGill

AP4 **A 3-D Mesoscale Wind Field Model and its Application for Emergency Planning at Nuclear Power Plants in Ontario**
H. Sahota, P.K. Misra, R. Bloxam and D. Rhee, Air Resources Branch, Environment Ontario

AP5 **The Results from a Meso-scale Model** M. Niewiadomski, University of Toronto, Toronto, Ontario

AP6 **Dose Response for Selected Environmental Air Pollutants: Results from a Study on Runners** R.B. Urch, F. Silverman, P. Corey and R.J. Shephard, The Gage Research Institute, University of Toronto, Toronto, Ontario

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AP7 **Hamilton Air: Chemical Composition and Genotoxic Activity of Respirable Particulate and Organic Vapours** D.W. Bryant, C. Kaiser-Farrell and D.R. McCalla, Department of Biochemistry, McMaster University, Hamilton, Ontario

AP8 **Mutagenicity Studies and Risk Estimation of Complex Mixtures of Organic Airborne Contaminants** A.S. Raj and D.M. Logan, Department of Biology, York University, Downsview, Ontario

AP9 **In-Situ Monitoring of the Environment for Genotoxicity Levels Using Rodents** M. Petras, M. Vrzoc, S. Meddins, K. Hill and T. Sands, Department of Biological Sciences, University of Windsor, Windsor, Ontario

AP10 **Method Development for the Monitoring and Analysis of Odorous Organics in Ambient Air** C.C. Chan, L. Vainer and J.W. Martin, Mann Testing Laboratories Ltd., Mississauga, Ontario, and A. Szakolcai and B. Foster, Environment Ontario

AP11 **Gas Phase Analysis of Organic Compounds from Structural Domain Modulation within Flourescent Lipid Multilayers** U.J. Krull, R.S. Brown and K. Stewart, Department of Chemistry, Erindale Campus, University of Toronto, Mississauga, Ontario

AP12 **Atmospheric Measurements of Natural Hydrocarbons Using Gas Chromatography/Mass Spectrometry** H. Niki and B.H. Khouw, Department of Chemistry and Centre for Atmospheric Chemistry, York University, Downsview, Ontario

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AP13 **Utilization of Established Air Pollution Monitoring Networks in Ontario Following Nuclear Incidents** J.A. Slade and G. Laszlo, Radiation and Industrial Safety Branch, Atomic Energy of Canada Limited, Chalk River, Ontario

AP14 **A Re-Examination of Ontario's 24 Hour Ambient Air Quality Criterion for Hydrogen Fluoride** R.D. Jones and D.S. Harper, Air Resources Branch, Environment Ontario

AP15 **Production of Ozone-insensitive White Bean Varieties** T.E. Michaels, Department of Crop Science, University of Guelph, Guelph, Ontario

AP16 **Efficacy of Film-forming Chemicals for Protecting Roadside Trees Against Salt Spray** C. Chong, Ministry of Agriculture and Food, Horticultural Research Institute of Ontario, Vineland Station, Ontario

AP17 **An Evaluation of the Problems of Particulate Emission from the Wood Products Industry** M.F. Lepage and A.E. Davies, Rowan Williams Davies & Irwin Inc., Guelph, Ontario

AP18 **Relationship of Sugar Maple Decline and Corresponding Chemical Changes in Xylem Sap Carbohydrates, Micronutrients and Trace Elements** S.N. Pathak, T. Hutchinson and D.N. Roy, Department of Forestry, University of Toronto, Toronto, Ontario

AP19 **Identification of Long Range Aerosol Sources at the Dorset Environment Station** J. Drake, A. Kabir and S. Vermette, Department of Geography, McMaster University, Hamilton, Ontario

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SESSION C: LIQUID AND SOLID WASTE RESEARCH

Oral Presentations

C1 **An Overview of Hydrogeological Aspects of Waste Disposal: Research Results and Implications** J. Cherry, Waterloo Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

C2 **Immiscible Liquids and Vapours in Soil: Recent Experiments on Transport and Control** G. Farquhar*, R. Bensen, D. Graham, E. McBean and B. Stickney, Dept. of Civil Eng., University of Waterloo, Waterloo, Ontario.

C3 **Effects of Increasing Amounts of Non-polar Organic Liquids in Domestic Waste Leachate on the Hydraulic Conductivity of Clay Liners in Southern Ontario** F. Fernandez* and R. M. Quigley, University of Western Ontario, London, Ontario.

C4 **Technology Review: Biological Treatment of Hazardous Landfill Leachates** J. Fein*, and P. Yu, Diversified Research Laboratories Ltd., Toronto, Ontario.

C5 **Phase Partitioning Kinetics at Industrial Waste Land Treatment Sites** D. Hockley and W. J. Snodgrass*, Beak Consultants, Toronto, Ontario.

C6 **Preliminary Assessment of a Microfiltration/ Reverse Osmosis Process for the Treatment of Landfill Leachate** T. A. Krug* and S. McDougall, Zenon Environmental Inc., Burlington, Ontario.

C7 **Anaerobic Treatment of Landfill Leachate** G. P. Vicevic*, B. J. Forrestal and A. Stevenson, Ontario Research Foundation, Clarkson, Ontario.

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Oral Presentations

C8 **The Origin and Distribution of Methane in the Alliston Sand Aquifer** R. Aravena*, J. Barker, M. Bliss and L. Wassenaar, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario.

C9 **The Carbon and Sulfur Cycle in Shallow Unconfined Aquifer Systems** L. I. Wassenaar*, R. Aravena, R. W. Gillham, J. Barker and P. Fritz, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario.

C10 **Determination of Organic and Inorganic Contaminants in the Welland River** I.D. Brindle*, A.W. Chu and X-f Li, Chemistry Department, Brock University, St. Catharines, Ontario.

C11 **Research and Development of Permanent On-site Solutions for Contamination of Groundwater at Waste Disposal and Industrial Sites in Canada** R.J. Rush, CANVIRO Consultants, Kitchener, Ontario.

C12 **The Role of Groundwater in Human Society** R.N. Farvolden, Waterloo Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

C13 **Dispersion of the Stouffville Landfill Plume** I. Proulx* and R.N. Farvolden, Waterloo Centre for Groundwater Research, University of Waterloo, Ontario.

C14 **Comparison of an Experimental Municipal Refuse Column Study with Landfill Field Test Cells** S. Pirani and D.W. Kirk*, Dept. of Chemical Engineering, University of Toronto, Toronto, Ontario.

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C15 **An Alternative to Incineration of Biomedical Waste: Hammermill/Chemical Decontamination** J. Manuel, Waste Management Branch, Environment Ontario.

C16 **Erosion of Landfill Covers** J. Cuthill*, Department of Land Resource Science, University of Guelph, Guelph, Ontario, and K. McKague, Ecologistics Ltd., Waterloo, Ontario.

C17 **Development of Backfill and Construction Application Guidelines for Ontario** M. Kelleher* and B. Whiffin, CANVIRO Consultants, Mississauga, Ontario.

C18 **Panel Discussion: Stemming the Rising Tide of Waste**
Moderator: D. Mackay, University of Toronto

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CP1 **Retractable Composite Absorbents for Environmental Clean-up** B. Gillies, E. Stubley, I. Treurnicht and L. Read, EcoPlastics Ltd. Willowdale, Ontario and O. Meresz, Laboratory Services Branch, Environment Ontario.

CP2 **Treatment and Disposal of Hauled Sewage Under ', 'Part VII, Environmental Protection Act** J.L. Smith, Oliver, Mangione, McCalla & Associates Limited, Nepean, Ontario.

CP3 **Factors Affecting the Concentration of Metal Ions in Municipal Refuse Leachate** G. Kosta, S. Pirani and D. Kirk, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario.

CP4 **Slow Rate Infiltration Land Treatment and Recirculation of Landfill Leachate in Ontario** R.A. McBride, A.M. Gordon, P.H. Groenevelt, T.J. Gillespie and L.J. Evans, Departments of Land Resource Science and Environmental Biology, University of Guelph, Guelph, Ontario.

CP5 **Establishing Vegetation on Erosion-prone Landfill Slopes in Ontario, Year Two** T.W. Hilditch and C.P. Hughes, Gartner-Lee Ltd., Markham, Ontario.

CP6 **Evaluating Groundwater Velocity in a Low-Permeability Fractured Shale** K.S. Novakowski and J.A. Cherry, Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

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CP7 **The Design and Evaluation of In-Situ Biorestoration Methods for the Treatment of Sludges and Soils at Waste Disposal Sites** K.L. Berry-Spark and J.F. Parker, Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario.

CP8 **Enhanced Biodegradation of Aromatic and Chlorinated Aliphatic Compounds in a Leachate-Impacted Aquifer** -D.W. Acton, M. Shaw, J.F. Barker, C.I. Mayfield and J.A. Cherry, Univeristy of Waterloo, Waterloo, Ontario.

CP9 **Waste Management Planning for Pharmaceutical Industry** R. Staris and R. Makhija, Trent University, Peterborough, Ontario.

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D1 Analytical Chemistry in a Regulatory Environment R. Kagel, Dow Chemicals, Midland, Michigan, U.S.A.

D2 Adaptation of Water Preconcentration Techniques Developed for PCDD Analysis to Other Target Organic Pollutants. E. Dowdall*, B.R. Hollebone, L. Brownlee and C. Shewchuk, Carleton University, Ottawa, Ontario.

D3 The Purpose and Significance of Ultratrace Analysis of Dibenzo-p-Dioxins: The Concept of Risk L. Brownlee* and B.R. Hollebone, Chemistry Department, Carleton University, Ottawa, Ontario.

D4 Procedures for the Analysis of 2,3,7,8-Substituted PCDD & PCDF Isomers and Other Target Compounds in Environmental Samples F.W. Karasek*, T.S. Thompson and K.P. Naikwadi, University of Waterloo, Waterloo, Ontario.

D5 The Closed-Loop Stripping Technique, Applied to Potable Water to Solve Taste and Odour Problems J.P. Palmentier*, D. Robinson and V. Taguchi, Laboratory Services Branch, Environment Ontario.

D6 Solid Supported Processes in Environmental Analysis J.M. Rosenfeld, Department of Pathology, McMaster University, Hamilton, Ontario.

D7 Synthesis and Use of Liquid Crystalline Polysiloxane Substrate in Capillary Column GC-MS for Isomer Specific Separation of Toxic Isomers of PCDD and PCDF K.P. Naikwadi* and F.W. Karasek, University of Waterloo, Waterloo, Ontario.

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D8 **Development of Mobile Infrared Spectroscopy for On-site Speciation of Organic Wastes** P. Yang* and J. Osborne, Laboratory Services Branch, Environment Ontario.

D9 **Mobile Laboratory: On the Development and Real World Application Aspects** D. Toner*, B. Dalton, D. Morse, K. Hom, P. Yang and J. Osborne, Laboratory Services Branch, Environment Ontario.

D10 **Regiospecific Synthesis of All Isomeric Nitrofluorenones and Nitrofluorenes by Transition Metal Catalyzed Cross Coupling Reactions** V. Snieckus*, T. Iihama, J.-m Fu and M. Bourguignon, University of Waterloo, Waterloo, Ontario.

D11 **Preparation of Heterocyclic Polynuclear Aromatic Compounds as Analytical Standards** E. Lee-Ruff*, B.E. George, F.J. Ablenas and Y.S. Chung, Department of Chemistry, York University, Downsview, Ontario.

D12 **Application of ICP Spectrometry in Health and Environment: A Case Study of Soil Ingested by Children** R. Barnes, University of Massachusetts, Amherst, Massachusetts, U.S.A.

D13 **Direct Sample Insertion into an Inductively Coupled Plasma for Atomic Emission and Mass Spectrometry** L. Blain* and E.D. Salin, Department of Chemistry, McGill University, Montreal, Quebec.

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D14 **Analysis of Germanium and Tin by Hydride Generation D.C. Plasma Atomic Emission Spectrometry: Application to Determinations of Germanium and Tin in Air Filters** I.D. Brindle*, B. Buchanan and X-c. Le, Brock University, St. Catharines, Ontario.

D15 **Use of the Hot Slurry Technique for Solid Sample Introduction for ICP-AES** L. Gervais* and E.D. Salin, Department of Chemistry, McGill University, Montreal, Quebec.

D16 **Advanced Technology for Destruction of Waterborne Organic Pollutants** H. Al-Ekabi* and M. Robertson, Nulite, A Division of Nutech Energy System Inc., London, Ontario.

D17 **Development of ACexpert 2: Implementation of an Expert System for Automated Metal Analysis by Atomic Absorption Spectroscopy** M.J. Stillman*, T.A. Cox and W.R. Browett, University of Western Ontario, London, Ontario.

D18 **Adaptation of Water Preconcentration Techniques of Trace Metal Detection** K.L. Singfield*, B.R. Hollebone, L.J. Brownlee, Dept. of Chemistry, Carleton University, Ottawa, Ontario, and P. Vijan, Environment Ontario.

D19 **Comparison of Various Leachate Extraction Procedures for the Characterization of Inorganics in Wastes** J.R. Kramer*, P. Brassard, J. Gleed and P.V. Collins, Department of Geology, McMaster University, Hamilton, Ontario.

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D20 **2,4-Dichlorophenoxylacetic Acid (2,4-D) Determination in Water, Urine and Soil Extracts by Enzyme Immunoassay (EIA) and Radioimmunoassay (RIA)**
J.C. Hall* and K. Krieg, Dept. of Environmental Biology, University of Guelph, Guelph, Ontario.

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DP2 **New Chemical Ionization Reagents Directed Toward Mass Spectrometric Analysis of Trace Organics** T.B. McMahon, K. Froese and C.E. Allison, Department of Chemistry and Guelph-Waterloo Centre for Graduate Work in Chemistry, University of ', 'Waterloo, Waterloo, Ontario.

DP3 **An Interrupted Segemented Flow Stream Microwave', 'Solid Sample Decomposition for ICP-AES** E.D. Salin And B. Liu, Department of Chemistry, McGill University, Montreal, Quebec.

DP4 **Solid Phase Extraction of PAH's From Drinking Water and Analysis of Chlorophenols and Phenoxy-acid Herbicides in Water** W.G. Craig and C.D. Hall, Paracel Laboratories Ltd., Nepean, Ontario.

DP5 **Automated Water Preconcentration Sampler for Dioxin Detection at the Parts Per Quadrillion Level** C. Shewchuk, B. Hollebone, L. Brownlee and E. Dowdall, Carleton University, Ottawa, Ontario, and R. Hunsinger, M. Uza, H. Tosine and S. Suter, Environment Ontario.

DP6 **Automated HPLC Method for Low Level Polynuclear', 'Aromatic Hydrocarbon (PAH) Analysis of Drinking Water** P.W. Crozier and C.D. Hall, Laboratory Services Branch, EnvironmentOntario.

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DP7 **Supercritical Fluid Extraction of Trace Organics From Solid Matrices** P. Kruus and R. C. Burk, Department of Chemistry, Carleton University, Ottawa, Ontario and G. Crawford, Laboratory Services Branch, Environment Ontario.

DP8 **Automated Sample Introduction and Pre-treatment', 'with Flow Injection ICP-ES** J. F. Hopper, F. Mo and D. W. Boomer, Laboratory Services Branch, Environment Ontario.

DP9 **Applications of Flow Injection Technology to ICP-MS** M. J. Powell, J. F. Hopper and D. W. Boomer, Laboratory Services Branch, Environment Ontario.

DP10 **Investigation of the In-Situ Acetylation Process and its Applicability to the Analysis of a Wide Range of Phenolic Compounds in Water** R. Lega, O. Meresz and M. Savu, Laboratory Services Branch, Environment Ontario.

DP11 **Robustness of the Student's T-test with Censored Environmental Quality Data** E. E. Creese, Creese Environmental Consulting, Waterloo, Ontario.

DP12 **Automation of Solid Supported Reactions by Robotics** J. M. Rosenfeld and E. Pevolinas, McMaster University, Hamilton, Ontario.

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SESSION E: ENVIRONMENTAL ECONOMICS

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E1 **Understanding Environmental-Economic Integration** P.A. Victor, VHB Research Ltd., Toronto, Ontario.

E2 **Economic Valuation Disparities and Environmental Policies** J.L. Knetsch, Economics Department, Simon Fraser University, Burnaby, British Columbia.

E3 **The Physco-social Impacts of Exposure to Environmental Contaminants in Ontario: A Feasibility Study** S.M. Taylor*, J. Frank, M. Haight, D. Streiner, S. Walter and N. White, McMaster University, Hamilton, Ontario.

E4 **Economic Assessments of MISA Regulations for Direct Industrial Dischargers in Ontario** O.E. Salamon* and J.A. Donnan, Policy and Planning Branch, Environment Ontario.

E5 **The Extra Strength Sewer Surcharge to Regulate Industrial Sanitary Waste Discharges** M. Fortin*, Ecologistics, Waterloo, Ontario, G. Zudovs, CANVIRO, and J. Donnan and G. Zegarac, Environment Ontario.

E6 **A Study of the Economic Factors Relating to the Implementation of Resource Recycling or Zero-Discharge Technologies for Heavy Metal Generating Industries in Canada** B. Fleet*, J. Kassirer, T. Burrell, T. Sanger, C. Small and B. Cardoza, University of Toronto, Toronto, Ontario.

E7 **Determinants of Participation in Solid Waste Source-Separation Programs in High-Rise Apartment Buildings** V.W. Maclaren, Department of Geography, University of Toronto, Toronto, Ontario.

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SESSION E: ENVIRONMENTAL ECONOMICS

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EP1 **The New Economics of Sustainable Development** R.Z. Rivers, Water Planning and Management Branch, Canada Centre For Inland Waters, Environment Canada, Burlington, Ontario.

EP2 **The Environmental Effects of Forestry Operations in Ontario: How Much Do We Know?** J.A. Dunster, Federation of Ontario Naturalists, Toronto, Ontario.